

**GEOGRAPHIC AND TEMPORAL VARIATION IN THE GENETIC MATING  
SYSTEMS OF PIPEFISH**

A Dissertation

by

KENYON BRICE MOBLEY

Submitted to the Office of Graduate Studies of  
Texas A&M University  
in partial fulfillment of the requirements for the degree of  
DOCTOR OF PHILOSOPHY

August 2007

Major Subject: Zoology

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## ABSTRACT

Geographic and Temporal Variation in the Genetic Mating Systems of Pipefish.

(August 2007)

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Chair of Advisory Committee: Dr. Adam G. Jones

Understanding the processes that govern mating behaviors is a fundamental goal of evolutionary biology and behavioral ecology. Population-level patterns of mate acquisition and offspring production, otherwise known as the genetic mating system, play a central role in the sexual selection on morphological and behavioral traits and may facilitate speciation. The central hypothesis of this research is that variation in environmental conditions, such as temperature, turbidity, and habitat, and demographic influences such as population density, sex ratios and temporal availability of mates, may limit mating and reproductive success in a predictive manner. Therefore the goal of this dissertation is to examine the contributions of geographic and temporal variation on the plasticity of the genetic mating system in two species of pipefish.

The first study examined whether meaningful variation in the genetic mating system exists between two natural populations of the dusky pipefish, *Syngnathus floridae*. Results of this investigation provide evidence that the genetic mating system differs among different geographic locations.

The second study considered the relative contributions of environmental conditions and population demographics on differences in the genetic mating system of dusky pipefish from five natural populations. The results of this investigation show strong trends for demographic and environmental factors to strongly influence the genetic mating system between populations.

The third study considered how variation in the number of available mates predicts the outcome of sexual selection during the course of a breeding season in the broad-nosed pipefish, *Sygnathus typhle*. The results of this study indicate a strong influence of the operational sex ratio on the genetic mating system.

In addition to these studies, a study was conducted to investigate whether phylogeographic relationships may be responsible for geographic variation in the genetic mating system of the dusky pipefish of pipefish. Mitochondrial DNA analysis does not substantiate subspecies designations for this species and microsatellite analysis show a clear pattern of isolation by distance.

Taken together, these studies significantly enhance the understanding of how mating systems are organized over broad environmental gradients and temporal/spatial scales and to the evolution of sexual selection on the whole.

## **DEDICATION**

To C. Adrian Phillips my best friend  
and Dr. Sue Cairns who taught me to appreciate  
a wing of a bat and a leg of a frog

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All aspects of this research were conducted following guidelines within the Texas A&M Animal Use Protocol 2004-227.

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## CHAPTER I

### INTRODUCTION

#### Background

In their seminal article, Emlen & Oring (1977) put forth the first unified theory of mating system evolution. This conceptual framework has provided the basis for various studies on the ecology and evolution of mating behavior in the decades since their original proposal. Emlen & Oring (1977) proposed that mating systems comprise various components of reproductive behavior, most notable of which are 1) the number of mates acquired, 2) the manner of mate acquisition, 3) the presence and characteristics of pair bonds, and 4) the patterns of parental care provided by each sex. Mating systems can be further divided into the social mating system and genetic mating system. The social mating system is primarily concerned with the formation of pair bonds and other behavioral interactions between potential mates and rivals (Jones & Avise 2001a). The genetic mating system, on the other hand, is concerned only with the number of mates acquired and the number of offspring produced by each breeding adult during one well defined breeding interval (Andersson 1994, Jones & Avise 2001). The genetic mating system is of particular interest to evolutionary biologists since the numbers of mates acquired and the numbers of offspring produced by each mate are directly related to sexual selection, or the competition for mates (Darwin 1859, 1871, Jones & Avise 2001, Shuster & Wade 2003). Sexual selection is a powerful selective process that

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This dissertation follows the style of *Molecular Ecology*.

drives the evolution of morphology and behaviors that are related to the competition for mates (Shuster & Wade 2003). The genetic mating system, therefore, has a profound influence on sexual selection within a species as well as contributing significantly to a species' life history strategy.

Two recent developments have brought mating system biology back to the forefront in scientific inquiry. The first development is the application of sexual selection theory to mating system biology by employing selection gradients to characterize the genetic mating system (Bateman 1948, Arnold & Wade 1984, Jones & Avise 2001, Jones et al. 2002, Shuster & Wade 2003, Jones et al. 2005). Selection gradients can then be compared across sexes, populations and breeding seasons in a statistically rigorous manner. The second development in mating system theory is the application of molecular techniques to accurately and unambiguously assign parentage to offspring. Studies that employ molecular parentage techniques have linked classical behavioral studies with direct measurements of sexual selection thereby providing a statistically rigorous framework for hypothesis testing (see Shuster & Wade 2003 for review). By combining and applying these two recent developments to genetic mating systems, we can now take advantage of powerful molecular-based techniques to calculate both mating and reproductive success while providing a theoretical framework for testing hypothesis-driven questions regarding mating systems.

*Measurement of sexual selection by means of Bateman's principles*

In *The Origin of Species* (1859) Darwin originally proposed the concept of sexual selection arising from inter- and intra-sexual competition for access to mates. However, direct measurement of the strength and direction of sexual selection has provided a challenge to modern research (Jones et al. 2002, Shuster & Wade 2003). In the time since Darwin, researchers have proposed several measures such as relative parental investment (Trivers 1972), the operational sex ratio (OSR, Emlen & Oring 1977) and the maximum potential reproductive rates of the sexes (Clutton-Brock & Vincent 1991) to evaluate sexual selection and mating system organization. None of these indices of sexual selection directly measure the intensity of selection, although some, like the operational sex ratio, certainly have dramatic effects on sexual selection (Kvarnemo & Ahnesjö 1996, Shuster & Wade 2003).

Bateman (1948) provided a theoretical framework for sexual selection by articulating three specific points now referred to as “Bateman's principles” (Arnold & Duvall 1994). Based on Bateman's original work, Arnold & Duvall (1994) recognized that 1) the sex experiencing the strongest sexual selection has higher reproductive success (measured in terms of the total number of offspring produced), 2) the sex experiencing the strongest sexual selection has a greater variance in mating success (measured in terms of number of mates), and 3) the slope of the regression relating reproductive success to mating success increases with the intensity of sexual selection (Arnold & Duvall 1994). Bateman's first principle measures the strength of selection arising from the standard variance in reproductive success also known as the opportunity



for selection or  $I$  [ $I = \text{variance}_{rs} / \text{mean}_{rs}^2$ ]. This measure can best be described as the theoretical maximum strength of selection that may act on a population in terms of offspring production (Crow 1958, 1962). Bateman's second principle measures the strength of sexual selection arising from the standard variance in mating success and is known as the opportunity for sexual selection,  $I_s$  [ $I_s = \text{variance}_{ms} / \text{mean}_{ms}^2$ ]. The opportunity for sexual selection indicates the maximum strength of sexual selection acting in a population (Wade & Arnold 1980). Arnold & Duvall (1994) proposed that the strength of sexual selection could be measured by the relationship between mating success and reproductive success, quantified by the Bateman gradient,  $\beta_{ss}$ . Since mating success and reproductive success must translate into increased fitness for individuals this relationship must be positive in order for sexual selection to operate (Jones et al. 2002). The Bateman gradient is the slope of the weighted regression line comparing reproductive success to mating success (Arnold and Duvall 1994). Finally the sex difference in opportunity for selection,  $I_{\text{males}}$ , [ $I_{\text{males}} - I_{\text{females}} = I_{\text{males}}$ ] has also been used as a measurement of sexual selection (Wade 1979; Wade & Arnold 1980; Wade 1995; Shuster & Wade 2003). The advantages of these variance related approaches are 1) they are directly related to Darwin's original hypothesis for sexual selection (Jones et al. 2004) and 2) they add a statistically rigorous component to compare the strength of sexual selection between sexes and among populations and species (Shuster & Wade 2003). A major disadvantage to applying Bateman's principles to natural populations is that the population would need to be small enough so that a majority of adults can be

sampled in order to accurately calculate the variation in mating success including the proportions of individuals that do not mate.

Recently, a renewed interest in mating system organization has established Bateman's principles as an appropriate method for comparing mating systems across populations and taxa (Bateman 1948, Arnold & Duvall 1994, Jones & Avise 2001, Shuster & Wade 2003, Jones et al. 2004, Jones et al. 2005, Mills et al. 2007). The modern iteration of Bateman's principles provides a rigorous and statistically quantitative measure of the potential for sexual selection (Jones & Avise 2001, Shuster & Wade 2003) and may lead to insights that help to explain observed patterns of diversity in animal mating systems. For example,  $I$  and  $I_s$  have been used to predict the outcome of competition between sexes in species with conventional sex roles including insects (Markow 2002), fishes (Downhower et al. 1987, Jirotkul 1999, Kelly et al. 1999, Lindström 2001), amphibians (Jones et al. 2002, Tennessen & Zamudio 2003), reptiles (Prosser et al. 2002), birds (Weatherhead & Boag 1997, Griffith et al. 1999, Richardson & Burke 2001, Webster et al. 2001) and mammals (Coltman et al. 1999, Galimberti et al. 2002). Additionally Bateman's principles have been applied to species that have alternative mating behaviors (Mills & Reynolds 2003, Shuster & Wade 2003), are obligate brood parasites (Woolfenden et al. 2002) and are sex role-reversed (Jones et al. 1999). A recent study by Jones et al. (2002) documents that two sexually selected traits in male rough-skinned newts, *Taricha granulosa*, are correlated to the direction and intensity of sexual selection by means of the Bateman gradient. This is the first study that made the definitive link of sexually selected traits and Bateman's principles thus

demonstrating the utility of such techniques. Also the application of Bateman's principles is useful to compare sexual selection between sexes, populations, species and higher taxa (Bateman 1948, Jones et al. 1999, Jones & Avise 2001, Shuster & Wade 2003). Despite the potential benefit of applying Bateman's principles to quantify sexual selection, they remain an underutilized proxy for sexual selection in natural populations.

*Other methods for calculating the genetic mating system*

Two additional variance methods, the index of resource monopolization (Ruzzante et al. 1996) and the Morisita index (Morisita 1962) have been recently introduced as alternative measures of the potential for sexual selection (Kokko et al. 1999, Fairbairn & Wilby 2001). The index of resource monopolization is defined as

$$Q = \frac{(\sigma^2 - \bar{x})}{(n\bar{x} - \bar{x})}, \text{ and the Morisita index is } I_\delta = n \left[ \frac{\sum_{i=1}^n x_i^2 - n\bar{x}}{(\bar{n\bar{x}})^2 - n\bar{x}} \right], \text{ where } n \text{ is the}$$

number of individuals,  $x_i$  is the value of the resource (e.g., mating success or reproductive success) for the individual  $i$ ,  $\bar{x}$  is the mean value across the  $n$  individuals, and  $\sigma^2$  is the variance. Fairbairn & Wilby (2001) advocated the application of Morisita's index for populations with female-biased sex ratios instead of  $I$  and  $I_\delta$ . The argument for these methods is that these measures based on Poisson distribution are not as sensitive to population means and therefore are a better metric of sexual selection. Neither of these techniques have gained wide acceptance for quantifying sexual selection to date and are heavily criticized for their redundancy (Jones et al. 2004, Mills et al. 2007).

Perhaps the major drawback to applying  $I_\delta$  and  $Q$  is a lack of connection to formal

selection theory based on Darwin's original concept of competition for mates (Jones et al. 2004, Jones et al. 2005).

### *Geographic variation in mating systems*

Only a handful of studies investigating variation in genetic mating systems on broad geographical scales have been conducted and they have not provided clear trends with respect to mating system organization among sites. For example, a few studies that investigated the degree of multiple mating have found little or no variation between geographically distant populations (Jones et al. 2001b, Goodisman et al. 2002). Another study discerned differences in mating success that are correlated with phenotypic variation in traits between populations such as male size in sailfin mollies (Trexler et al. 1997). Still other studies have provided evidence that there are significant differences between mating and reproductive success among distant populations, although the ecological factors responsible for such differences are not yet well understood (Weatherhead & Boag 1997, Griffith et al. 1999). Finally, only one model system has clearly shown a positive relationship between an ecological parameter (predation) and a mating system parameter, the degree of multiple insemination in Trinidadian guppies (Kelly et al. 1999, Bronikowski et al. 2002).

Despite the relative importance of environmental variation to other aspects of ecological and evolutionary biology, it remains a relatively understudied aspect of mating system organization. Few empirical studies have specifically tested how environmental factors influence the mating system of species (Turner & McCarty 1998).

Because of this, the study of biotic and abiotic factors that may constrain mating and reproductive success that do not specifically pertain to the acquisition of mates are often ignored and therefore little understood. We do know, however, that environmental factors may set limits on reproductive success. For example, temperature (Ahnesjö 1995, Kvarnemo 1997, Fischer et al. 2003), resource abundance (Kvarnemo 1997, Turner & McCarty 1998), habitat structure and fragmentation (Turner & McCarty 1998, Aguilar & Galetto 2004), parasite load (Fitze et al. 2004), resource competition (Martin & Martin 2001) and predation (Bronikowski et al. 2002) all may influence variation in the number of offspring produced by each mate. Environmental factors may also influence the mating success of a population. For example, length and synchrony of the breeding season (Emlen & Oring 1977, Shuster & Wade 2003, Spottiswoode & Møller 2004), local population density (Lloyd 1967, Griffith et al. 2002, Prohl 2002), the operational sex ratio (Emlen & Oring 1977, Kvarnemo & Ahnesjö 1996, Prohl 2002) and predation-risk (Kelly et al. 1999) have all been shown to affect the mating success of particular populations. The next logical step in mating system theory is to correlate sources of environmental variation with differences in mating system organization.

#### *Temporal variation in mating systems*

Emlen & Oring (1977) proposed that males compete with one another for access to females and male reproduction is thus limited by the spatial and temporal availability of sexually receptive females. Extending this idea further, Emlen & Oring (1977) suggested that the temporal pattern of female receptivity to mating may further limit a

male's opportunity to monopolize mates. For example, if all females become receptive at one particular time, then a male's ability to monopolize many females simultaneously may be reduced. Therefore the degree of mating synchrony, as well as the spatial dispersion of females, may play an important role in the intensity of sexual selection and the mating system. Few studies have specifically addressed how temporal variation in receptivity to mates may affect the mating system. Among isopods that dwell in the spongocoels of marine sponges, asynchrony in temporal receptivity of female isopods increases  $I_s$  (Shuster & Wade 2003). In at least two species of sex-role reversed pipefish, asynchrony in temporal receptivity of males to females has been hypothesized to decrease the OSR thereby increasing female competition for mates during the breeding season (Vincent et al. 1994, Vincent et al. 1995).

#### *Dissertation justification*

The justification for this dissertation is to resolve the role of phylogenetic history, environmental variation, population density and temporal variation on the organization of genetic mating systems. The central hypothesis of this research is that variation in environmental conditions, population density, sex ratios and temporal availability of mates may limit mating and reproductive success in a predictive manner. I will quantify the genetic mating system by applying Bateman's principles to draw comparisons between different sexes, populations and time periods (Bateman 1948, Arnold & Wade 1984). By investigating ecological parameters that may affect the local mating system, we may gain valuable insight into how species may modify their mating behavior in

response to environmental change. Details of ecological relevance, these studies take the next logical step in filling the gap of knowledge as to how ecological factors may affect mating systems. Thus, results from these studies will significantly enhance our understanding of how mating systems are organized over broad environmental gradients and temporal/spatial scales.

### *Study species*

To test these hypotheses, I will take advantage of the well documented genetic mating system of two sex-role reversed, polygynandrous pipefish, *Syngnathus floridae* and *S. typhle* (Jones & Avise 1997b, Jones et al. 1999). Like other members of the Genus *Syngnathus*, these species have male pregnancy characterized by the depositing of unfertilized eggs by the females into a specialized brood pouch on the male's ventral surface. Males fertilize the eggs and then provide all parental care to offspring until birth. These species are ideally suited to this type of inquiry for several reasons: 1) they are intimately associated with their natural seagrass habitat, 2) they have a broad geographical distribution that spans a wide range of ecological settings that vary with respect to temperature, habitat and community structure, 3) local population density and sex ratios vary spatially and temporally, and 4) mating behavior can easily be characterized by employing powerful molecular markers that have already been developed for these species (Jones & Avise 1997b, Jones et al. 1999).

### *Chapter outline*

In **CHAPTER II**, I explore the biogeographic phylogenetic relationship between Atlantic and Gulf of Mexico populations of *S. floridae* to explore how patterns of gene flow affect the distribution and evolutionary history of this species. To accomplish this goal, I use both nuclear and mitochondrial molecular markers to reconstruct phylogenetic relationships between populations. I use six microsatellite markers to construct an  $F_{ST}$  matrix on individuals from five populations (two Atlantic, three Gulf of Mexico) and sequence a 394bp fragment of the mitochondrial *cytochrome b* gene from 10 populations found throughout their continental US range.

In **CHAPTER III**, I compare the genetic mating system from a population of *S. floridae* from the Gulf coast of Florida to a population from the Atlantic coast of Virginia. To accomplish this goal, I collected adult populations from these two populations in 2003 and I investigated adult sex ratios, operational sex ratios, population densities, and the size of the adult population from each site to investigate their effect on the genetic mating system.

In **CHAPTER IV**, I explore the influence of population demographics (population density, operational sex ratio, adult sex ratio), and several environmental factors (daily and yearly water temperature, turbidity, salinity and seagrass height, density and biomass) on the genetic mating system of five populations *S. floridae*.

In **CHAPTER V**, I investigate how the availability of mates may affect the mating system of *S. typhle*. *Syngnathus typhle* undergo three semi-synchronous breeding events during a summer breeding season. At the start of the breeding season, males and



females arrive in shallow seagrass beds at roughly the same time, thus limiting any particular individual from monopolizing all mates. Because males' receptivity to females is constrained by the length of male pregnancy, females become in excess as the breeding season progresses, thereby increasing female competition for males.

In **CHAPTER VI**, I summarize the results of each study and will draw comparisons between each study with emphasis on their significance to mating system evolution, evolutionary biology and science in general.

**CHAPTER II**

**GEOGRAPHIC VARIATION IN THE GENETIC MATING SYSTEM OF THE**

**DUSKY PIPEFISH, *SYNGNATHUS FLORIDAE*.**

**I: PHYLOGEOGRAPHY**

**Introduction**

A fundamental goal of evolutionary research is to explain geographic patterns of variation among populations and species. Phylogeography, the study of patterns of genetic variation in nature through the reconstruction of genealogies, has played a major role in modern attempts to understand geographic patterns of biodiversity (Avice 2000). Phylogeographic studies have revealed historical patterns of population range expansions and bottlenecks and have clarified the extent to which populations are connected to one another by contemporary gene flow (Avice 2000). Comparisons of phylogeographic data across taxa have also revealed several naturally occurring barriers to gene flow (Avice 2000, Avice 2004). Perhaps the most famous natural barrier to gene flow is Wallace's Line, named after Sir Alfred Russel Wallace, who first noted distinct avian assemblages west of the island of Bali in the Malaysian Archipelago (Wallace 1869). Although the mechanism responsible for the divergent faunal assemblages were unclear to Wallace at the time, it was later determined that the Malaysian Archipelago was divided by a deep ocean trench and faunal assemblages on either side of the trench had extensive historical land connection (Voris 2000, Hall 2002).

Among the first studies that highlighted molecular support for such a barrier to gene flow were those conducted by Avice and colleagues (see Palumbi 1994, Hare &

Avise 1998, Avise 2000, 2004 for review). Using a variety of molecular markers, they documented a strong genetic discontinuity between populations of a variety of Southeastern United States maritime taxa. This biogeographic split separates species into Atlantic and Gulf of Mexico populations with the break roughly occurring on the mid-Atlantic coast of Florida. Similar to Wallace's line, there is no obvious physical barrier that characterizes this natural interruption to gene flow. It appears that this multi-species concordance in phylogeography is the result of historical vicariant events in this region. The prevailing hypothesis for the geographic patterns of genetic variation in this region is that populations were isolated after the last glacial maxima in the Pleistocene (Avise 2000). For instance, Reeb & Avise (1990) suggested that populations of the oyster, *Crassostrea virginica*, have been isolated during periods of low sea level in the Pleistocene. Populations of marine species across the northern portion of Florida may have also been connected much earlier during the Miocene via the Suwannee Seaway (Cunningham et al. 1991). Ecological conditions and temperature gradients may also provide a mechanism for the maintenance of phylogenetic divergence because a faunal transition zone occurs at this location separating temperate species of the north Atlantic from southern subtropical species (Avise 2000). Strong oceanic currents such as the Gulf Stream or the Gulf Loop Current provide one avenue of gene flow and may transport larvae and juveniles hundreds of kilometers, although the success rate of long distance dispersers may be small (Palumbi 1994).

The purpose of this study is to document the phylogeographic distribution of the dusky pipefish along the continental United States shoreline to test the hypothesis that

patterns of gene flow in this coastal fish are consistent with the biogeographical split between the Atlantic Ocean and the Gulf of Mexico. The dusky pipefish, *Syngnathus floridae* (Jordan & Gilbert), occurs in the Western Atlantic Ocean from the Chesapeake Bay south to Seabrooks Beach, South Carolina, Bermuda, the southeastern Florida coast south to Panama, the Gulf of Mexico, and the Caribbean (Herald 1965, Dawson 1982, Fig. 2.1). On the continental U.S., there is a break in the distribution of *S. floridae* between Seabrooks Beach SC and Fort Pierce, FL (Dawson 1982), making this species a particularly good candidate to show a pattern of genetic variation consistent with the observations for other marine species from the same region.

Because the dusky pipefish displays substantial among-population variation in body size, coloration, and meristic characters, such as fin ray, trunk ring and tail ring counts, throughout its range, there has been some debate with respect to subspecies designations in this pipefish species. A preliminary survey by Herald (1962), based on morphological characteristics and geographic distribution, designated *S. floridae* a polytypic species consisting of four subspecies. In Herald's (1962) scheme, the subspecies *S. f. hubbsi* ranged from the Chesapeake Bay (Plum Point, Maryland) to South Carolina (Seabrooks Beach). The second subspecies, *S. f. floridae*, occurred throughout the Gulf of Mexico from the west coast of Florida to Corpus Christi, Texas. The third subspecies, *S. f. mckayi*, included populations from Biscayne Bay, Florida through the Florida Keys and likely occurred in parts of the Caribbean, including the Bahamas, but records were scarce in the Caribbean. The fourth subspecies in Herald's classification (1962), *S. f. nesiotes*, was proposed to occur only in Bermuda. After a

more complete and thorough review of existing collections of *S. floridae*, Dawson (1982) doubted the existence of such a species complex. Although he documented variation in meristic traits among different populations of *S. floridae*, he suggested that perceived differences between populations could have resulted from undetermined ecological influences (Dawson 1982). He also suggested that gene flow between populations was likely as a consequence of dispersal of juveniles drifting in floating vegetation.

To investigate geographic patterns of genetic variation in the dusky pipefish, two different methods of estimating population structure and phylogeography were employed. First, microsatellite data were used to examine population subdivision via  $F_{ST}$ ,  $R_{ST}$ , and a Bayesian cluster analysis. Second, mitochondrial DNA (*mtDNA*) sequence data from the *cytochrome b* gene were used to estimate a haplotype network and to reconstruct the phylogeny of the dusky pipefish using both parsimony and maximum likelihood techniques.

## **Materials and Methods**

### *Sample collection*

Adult *S. floridae* were collected from 10 locales within their continental U.S. distribution during the summers of 2003-2006 (Table 2.1, Fig. 2.1). Fishes were collected from shallow seagrass beds using a 3m hand-drawn seine net with a 3.2mm mesh or trawled by boat using a 5m trawl with a 3mm mesh at a rate of approximately 1.8-2.2 km/hr. All fishes were sacrificed in the field by severing their spinal columns

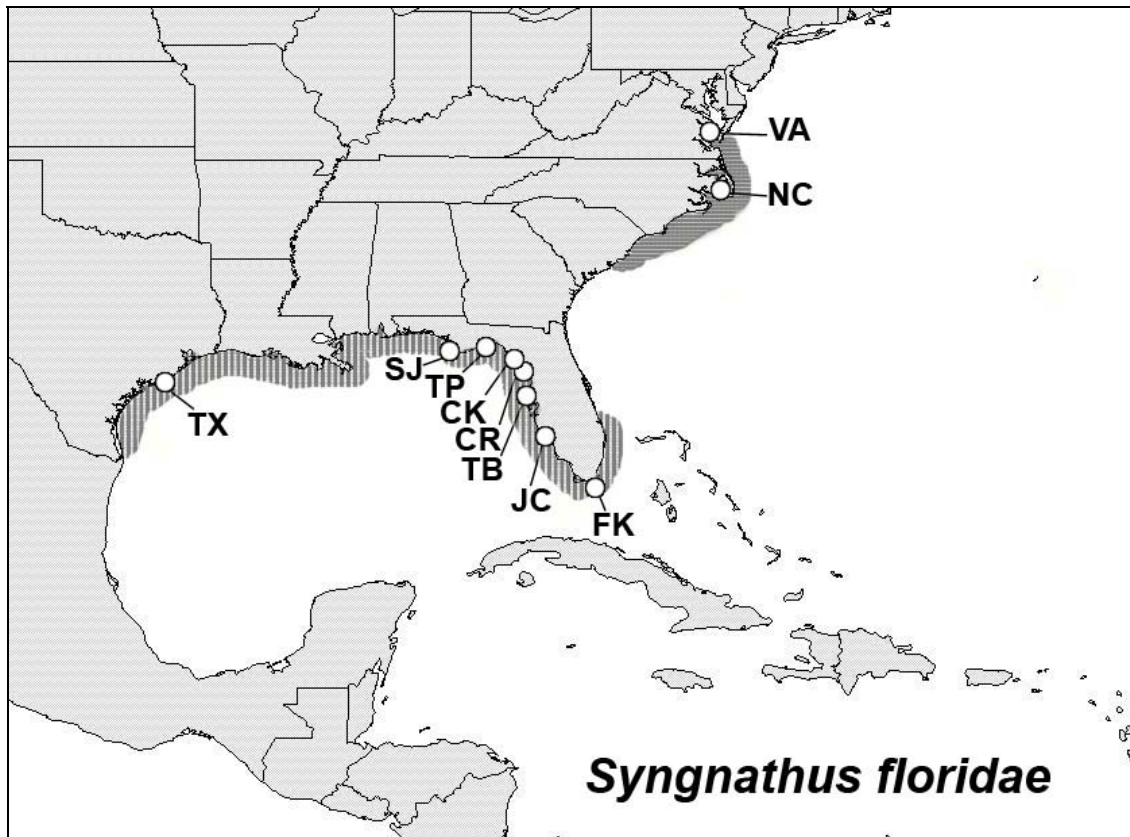


Figure 2.1. Map of the distribution of *Syngnathus floridae* within the continental US (shaded areas). Collection sites (circles) correspond with locations given in Table 2.1. No substantiated records for the species exist between Seabrooks Beach, South Carolina and Fort Pierce, Florida (Dawson 1982).

above the operculum. Whole fish either were immediately preserved in 95% EtOH in the field or were frozen on ice and transferred to 95% EtOH upon return to the laboratory. Total DNA was isolated from fin tissue using a Gentra Puregene® Cell Tissue Kit for microsatellite and mitochondrial analyses.

#### *Microsatellite markers*

Microsatellite loci were assayed in five populations of *S. floridae* that had sufficiently large samples sizes for meaningful analysis ( $n > 30$ ). The five populations examined consisted of two from the Atlantic Coast (Virginia, North Carolina), two from the Eastern Gulf of Mexico (Tampa Bay, FL, St. Joseph Bay, FL) and one from the Western Gulf of Mexico (Texas). A total of five variable microsatellite markers isolated from other species were used in this analysis (Jones & Avise 1997a, Jones et al. 1999). A sixth microsatellite, *Scov5* (*Scov5 forward* 5'-CGATAAGTGAGAGAGAGG-3', *Scov5 reverse* 5'-CACCGTGGGTTCAACTTTG-3') that amplified a AGC repeat motif, developed for *S. scovelli* was also used.

Microsatellite markers were amplified using polymerase chain reaction (PCR) conditions modified from Jones & Avise (1997b) and Jones et al. (1999). We used annealing temperatures of 54°C for *Micro11.1* and *Micro22.3*, 55°C for *Typh12* and 56°C for *Micro25.10*, *Micro25.22* and *Scov5*. All thermal cycling ran for 36 cycles, except that for *Micro11.1*, which ran for 40 cycles to yield sufficient PCR product for analysis. Primers were labeled with a fluorescent dye, multiplexed and sized on an ABI 3730 Genetic Analyzer (Applied Biosystems, Foster City, CA) by the Nevada Genomics

Table 2.1. Sites, abbreviation (abb), date(s) of collection, number of adults collected and GPS coordinates of collection of *Syngnathus floridae*.

Site	Abb	Date		GPS Coordinates (N, W)
<b>ATLANTIC</b>				
<i>Mobjack Bay, VA</i>	<b>VA</b>	8/03	241	37°1842', 76°2475'
<i>Morehead City, NC</i>	<b>NC</b>	7/04	82	34°4334', 76°7619'
<b>GULF OF MEXICO</b>				
<i>Spanish Harbor Bridge, FL</i>	<b>FK</b>	6/04	3	24°6497', 81°3169'
<i>Jug Creek Shoal, FL</i>	<b>JC</b>	7/06	4	26°7069', 82°1981'
<i>Tampa Bay, FL</i>	<b>TB</b>	6/04-7/04	126	28°0307', 82°4756'
<i>Crystal River, FL</i>	<b>CR</b>	8/06	2	28°8323', 82°8502'
<i>Cedar Key, FL</i>	<b>CK</b>	8/06	2	29°1354', 83°0987'
<i>Turkey Point Shoal, FL</i>	<b>TP</b>	8/05, 7/06, 8/06	12	29°8867', 84°5023'
<i>St. Joseph Bay, FL</i>	<b>SJ</b>	7/03-8/03	99	29°4776', 85°1824'
<i>Aransas Pass, TX</i>	<b>TX</b>	8/05, 7/06	100	27°8806', 97°1019'



Center (Reno, NV). All microsatellite fragment analysis was accomplished using Genemapper<sup>®</sup> software (Applied Biosystems, Foster City, CA).

Microsatellite data were analyzed with GENEPOP version 3.4 (Raymond & Rousset 1995) to test for Hardy-Weinberg equilibrium (Fisher's exact test) and for genotypic disequilibrium for pairs of loci within the population (Fisher's exact test). Global and population pairwise  $F_{ST}$  and  $R_{ST}$  values were calculated with SPAGeDi version 1.2 (Hardy & Vekemans 2002). Permutation tests (10,000 iterations) were applied to global and pairwise  $F_{ST}$  and  $R_{ST}$  values to test for departures from the null hypothesis of panmixia in SPAGeDi. To evaluate whether mutation played a significant role in population differentiation, an allele size permutation test was conducted by using SPAGeDi with 10,000 permutations of allele size for each population pair. A Bonferroni correction was implemented to correct for multiple comparisons for all above tests wherever necessary.

A Mantel's test, implemented in the ISOLDE program within GENEPOP, was used to examine the relationship between geographic distance (natural log transformed) and divergence in allele frequencies ( $F_{ST}/1 - F_{ST}$ ) among sites. A 100km maximum for immigration was assumed for immigration between populations for the Mantel's test. Although juveniles have been found in floating vegetation in major ocean currents (Dawson 1982), and thus may represent gene flow between populations, a 100km maximum is likely much larger than the maximum distance for migration of breeding adults. This analysis was run with both 100km and 1000km as a minimum distance

between samples to investigate the robustness of the effect of migration on isolation by distance.

The Bayesian analysis program STRUCTURE version 2.1 (Pritchard et al. 2000) was used to infer population structure and to calculate the probability of assigning individuals to parent populations from the five sampled populations. The program was first run using the admixture model that assumes all individuals potentially have mixed ancestry and assigns each individual to a designated population ( $K$ ). Ten independent runs, incorporating burn-ins of  $10^5$  Monte Carlo Markov chain (MCMC) replicates followed by  $10^6$  replicates of data collection were performed with admixture model program defaults for  $K$  fixed at 1-6 populations. A second model that incorporates information regarding the home population of each individual was executed using run, burn-in, MCMC parameters and program defaults similar to the admixture model. The probability of recent immigration to each population ( $\nu$ ) was fixed at 5% for both the admixture and no admixture models.

#### *mtDNA markers*

A 1201 bp fragment of the mitochondrial *cytochrome b* gene was amplified using primers L14725 (Pääbo et al. 1991) and H15926 (Wilson et al. 2001) on a minimum of 16 randomly chosen individuals for each heavily sampled locality. When fewer than 16 individuals were sampled at a particular locality, all individuals were sequenced.

Fragments were amplified by PCR in 30uL volumes containing 3μl of 10X buffer, 3μl of dNTPs (2μM), 1.8ul of  $MgCl_2$  (25mM), 1.5 μl of each primer (10uM), and 0.5 μl of Taq

(1Unit/ $\mu$ l). The thermal profile for amplification of both fragments consisted of an initial denaturation for 5 min at 96°C, followed by 40 cycles of 94°C (1 min), 48°C (1 min), 72°C (1 min) and a final extension at 72°C for 4 min. PCR products were purified with a Qiagen quick prep PCR cleanup kit and sequenced with the L14725 primer by the Nevada Genomics Center (Reno, NV) on an ABI 3730 DNA Analyzer. Sequences were proofread and first aligned with CLUSTALW (Chenna et al. 2003). Alignments were verified by eye using the program BIOEDIT (Hall 1999). Several other species from the genus *Syngnathus*, including *S. louisianae*, *S. scovelli*, *S. fuscus* and *S. leptorhynchus*, were chosen as outgroups, and *cytochrome b* sequences were obtained from Genbank (Genbank accession numbers: AF356056, AF356064, AF356068, AF356070; Wilson et al. 2001). Poorly amplified and truncated sequences were removed and all sequences were trimmed to a 394 bp fragment prior to final phylogenetic analysis.

Gene diversity and nucleotide diversity statistics were calculated using ARLEQUIN version 2.1 (Schneider et al. 2000). A parsimony-based haplotype network was constructed with the program TCS version 2.1 (Clement et al. 2000) using program defaults with and without *S. louisianae* as an outgroup. TCS was first run using the outgroup *S. louisianae* and failed to link with *S. floridae* within the limits of 95% confidence limits of parsimony (10 mutational steps). It was then determined that *S. louisianae* could be linked to *S. floridae* via 93% parsimony (10 mutational steps) and the outgroup was then removed and the analysis was rerun excluding *S. louisianae*.

Common haplotypes were removed from the data set using COLLAPSE version 1.2 (Posada 2007) prior to phylogenetic reconstruction. Phylogenetic relationships were

reconstructed using both Parsimony and Maximum-likelihood methods. Parsimony was performed using PAUP\* version 4.02b10 (Swofford 2003) with 100 heuristic search bootstrap replicates (1000 random addition sequences per search with the MullTrees option and TBR branch swapping). Maximum-likelihood was carried out using 500 bootstrap replicates of data collection using PhyML version 2.4.4 (Guindon et al. 2005), a BIONJ starting tree, and a GTR+I+ $\Gamma$  model of nucleotide substitution found with MODELTEST version 3.8 (via the MODELTEST Web Server) (Posada & Crandal 1998).

## Results

### *Microsatellite analysis*

All six microsatellite loci were polymorphic, with from six to 73 alleles segregating per locus across the five populations (Table 2.2). Fisher's exact tests indicated no significant departures from Hardy-Weinberg equilibrium among loci and among population after applying a Bonferroni adjustment (Rice 1989). Tests of linkage disequilibrium among loci were non-significant, supporting independent assortment of these microsatellite loci.

Significant population differentiation was evident from both pairwise and global  $F_{ST}$  and  $R_{ST}$  values in all pairs of populations except SJ and TB (permutation test  $P < 0.0017$  after Bonferroni adjustment, Table 2.3). To assess whether mutation had a significant effect on population differentiation, an allele size permutation test was applied. In all population pairs, the multilocus  $pR_{ST}$  values did not differ from  $R_{ST}$  suggesting that stepwise mutations are not a primary source of allelic variation among

populations (Hardy et al. 2003). A Mantel's test showed a positive relationship between geographic distance and global  $F_{ST}$  values (1000 permutations,  $R^2 = 0.71$ , 9 d.f.,  $P < 0.003$ ; Fig. 2.2). Different migration distances of 100km and 1000km did not affect the results of the linear regression.

The admixture model as implemented by STRUCTURE returned similar posterior likelihood probabilities for two ( $K=2$ ; Atlantic/Gulf split) and three ( $K=3$ ; Atlantic/Eastern Gulf/Texas) and had non-symmetric  $F_{ST}$  designations for each respective reconstructed parent population. Therefore, the three Gulf of Mexico populations were analyzed with the admixture model excluding two Atlantic populations with  $K$  fixed at 1-4 populations. However after this analysis was performed, no support for a Gulf of Mexico split existed and STRUCTURE was rerun to calculate posterior likelihood probabilities of individuals from known populations for  $K=2$  using the no admixture model. The results of these analyses suggest that there are two parent populations for *S. floridae* congruent with a genetic split between Atlantic and Gulf of Mexico populations (Fig. 2.3).

Table 2.2. Microsatellite data comparing the number of alleles ( $A$ ), sample sizes ( $N$ ), observed and expected heterozygosity ( $H_O$  and  $H_E$  respectively) between adult *Syngnathus floridae* collections.

Locus	$A$		VA	NC	TB	SJ	TX
<b>Micro11.1</b>	73	$N$	85	77	124	91	103
		$A$	33	34	52	44	34
		$H_O$	0.882	0.844	0.919	0.890	0.913
		$H_E$	0.877	0.902	0.968	0.956	0.943
<b>Micro22.3</b>	31	$N$	84	71	126	93	101
		$A$	19	20	26	24	25
		$H_O$	0.798	0.915	0.8927	0.892	0.960
		$H_E$	0.833	0.901	0.940	0.931	0.915
<b>Micro25.1</b>	17	$N$	31	31	26	29	26
		$A$	13	13	12	13	11
		$H_O$	0.839	0.806	0.923	0.724	0.885
		$H_E$	0.883	0.883	0.866	0.840	0.860
<b>Micro25.22</b>	31	$N$	76	78	122	92	103
		$A$	17	18	26	22	22
		$H_O$	0.882	0.872	0.918	0.946	0.835
		$H_E$	0.890	0.883	0.930	0.923	0.904
<b>Scov5</b>	8	$N$	28	31	23	25	31
		$A$	5	4	5	6	5
		$H_O$	0.821	0.516	0.652	0.720	0.742
		$H_E$	0.677	0.651	0.663	0.705	0.694
<b>Typh12</b>	6	$N$	31	29	27	27	31
		$A$	4	3	4	5	2
		$H_O$	0.258	0.310	0.556	0.407	0.097
		$H_E$	0.389	0.472	0.576	0.530	0.152

Table 2.3. Population pairwise  $F_{ST}$  and  $R_{ST}$  estimates (below diagonal,  $F_{ST}$  appears in bold) versus distance (km, above diagonal) for the five populations examined with microsatellite loci. Global  $F_{ST}$  and  $R_{ST}$  values were 0.026 and 0.040 respectively. All estimates are significant after sequential Bonferroni (Rice 1989) unless otherwise noted.

	VA	NC	TB	SJ	TX
VA	---	<b>415</b>	<b>2213</b>	<b>2560</b>	<b>3664</b>
NC	<b>0.001</b> 0.005	---	<b>1798</b>	<b>2145</b>	<b>3249</b>
TB	<b>0.029</b> 0.086	<b>0.017</b> 0.042	---	<b>347</b>	<b>1451</b>
SJ	<b>0.028</b> 0.061	<b>0.018</b> 0.023	<b>-0.001</b> <sup>NS</sup> <b>-0.002</b> <sup>NS</sup>	---	<b>1104</b>
TX	<b>0.054</b> 0.041	<b>0.051</b> 0.021	<b>0.037</b> 0.057	<b>0.029</b> 0.032	---

<sup>NS</sup> = non significant after sequential Bonferroni.

### *mtDNA analysis*

Both gene and nucleotide diversity estimates were lowest at the westernmost (TX) and northernmost (VA, NC) extent of *S. floridae*'s range in the continental United States (Table 2.4). High levels of gene and nucleotide diversity throughout the eastern Gulf of Mexico suggest high levels of gene flow among these populations, although caution should be exercised in populations where the number of individual samples is low. The highest nucleotide diversity and genetic divergence were encountered in the three samples from the Florida Keys (FK) and may represent gene flow from Caribbean and south Atlantic populations of dusky pipefish.

The TCS analysis identified 28 unique haplotypes of *S. floridae* with the limits of 95% parsimony (Fig. 2.4). For the most part, no major genetic break between Atlantic and Gulf populations was found. However, a single nucleotide position displayed a clear distinction between Atlantic and Gulf populations. The nucleotide substitution was a silent 3<sup>rd</sup> position transition (T→C) at nucleotide position 374 in the *cytochrome b* sequence (Fig. 2.4). All individuals in the Texas population were found to have C in this position and all Atlantic (Virginia and North Carolina) populations were fixed for T. Sites throughout the eastern Gulf of Mexico had both character states present (Fig. 2.4). The TCS haplotype network was characterized by a general absence of reticulation, except for one sequence from Tampa Bay, which displayed affinities with both Cedar Key, Florida, and North Carolina samples. Linkage to the outgroup *S. louisianae* was



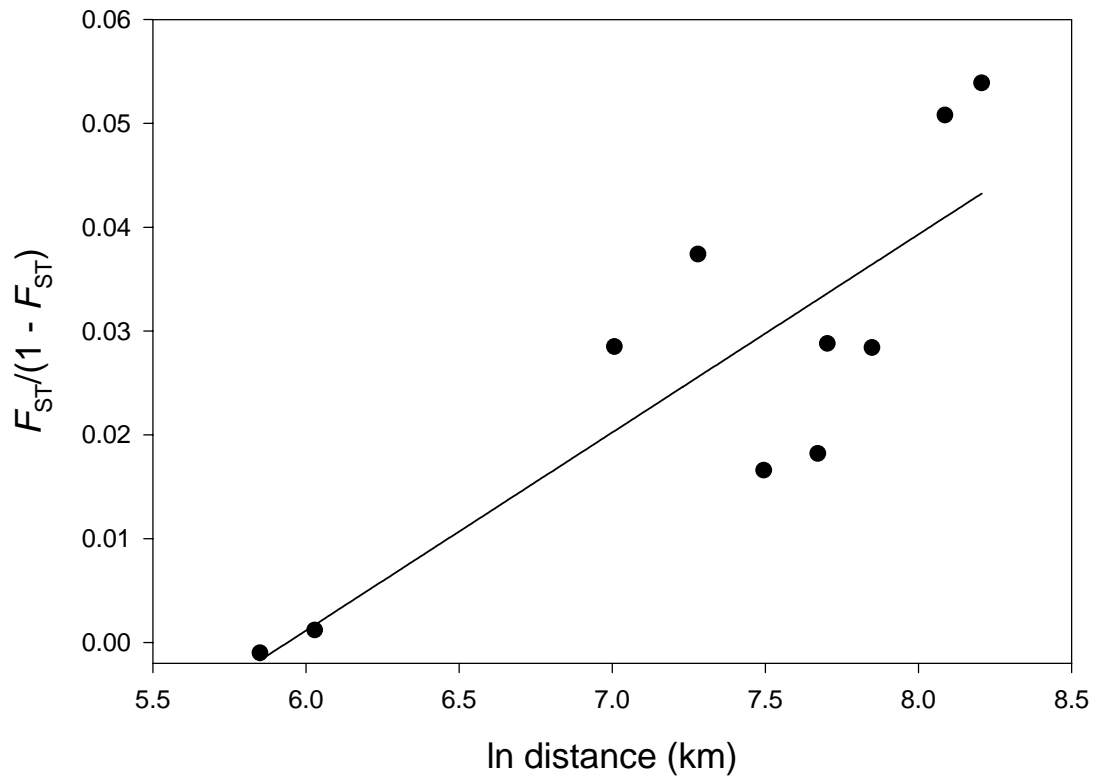


Figure 2.2. Relationship between genetic divergence ( $F_{ST}$ ) and geographic distance. Each point represents a single pairwise comparison between the five populations. This relationship is significant (Mantel Test, 10,000 permutations:  $R^2 = 0.71$ , 9 d.f.,  $P < 0.003$ ).

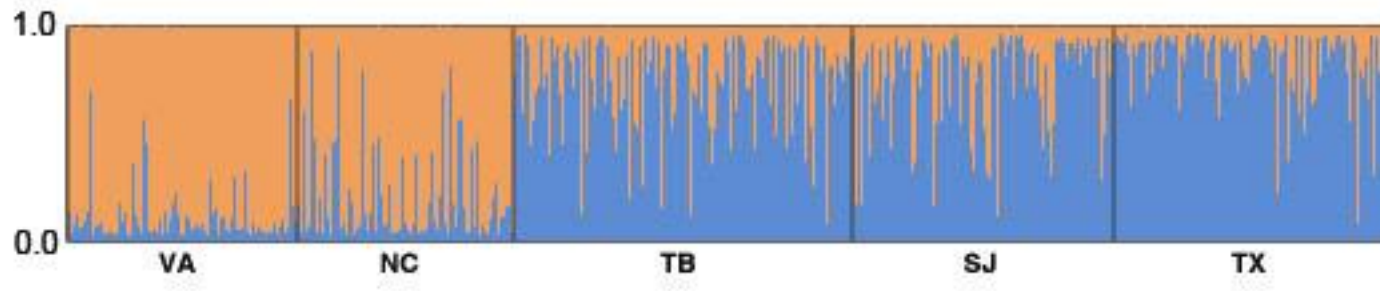


Figure 2.3. Results of Bayesian population structure for microsatellite data indicating two parent populations ( $K = 2$ ) shown in orange (Atlantic), and blue (Gulf of Mexico). Individual probabilities of assignment are shown on the y-axis and are grouped by parent populations calculated by the admixture program in STRUCTURE 2.1 (Pritchard et al. 2000).

Table 2.4. Statistics for *mtDNA* data. Number of individuals sequenced per population (*n*), number of unique *mtDNA* haplotypes, gene diversity and nucleotide diversity statistics are listed for each population.

<b>Site</b>	<b>n</b>	<b><i>mtDNA</i> haplotypes</b>	<b><i>mtDNA</i> gene diversity (<math>\pm</math> SD)</b>	<b><i>mtDNA</i> nucleotide diversity (<math>\pi</math>)(<math>\pm</math> SD)</b>
<b>VA</b>	13	3	$0.295 \pm 0.156$	$0.0008 \pm 0.0010$
<b>NC</b>	15	5	$0.476 \pm 0.155$	$0.0017 \pm 0.0015$
<b>FK</b>	3	3	$1.000 \pm 0.272$	$0.0288 \pm 0.0225$
<b>JC</b>	4	4	$1.000 \pm 0.177$	$0.0051 \pm 0.0046$
<b>TB</b>	13	12	$0.987 \pm 0.035$	$0.0071 \pm 0.0045$
<b>CR</b>	2	2	$1.000 \pm 0.500$	$0.0076 \pm 0.0087$
<b>CK</b>	2	2	$1.000 \pm 0.500$	$0.0025 \pm 0.0036$
<b>TP</b>	10	6	$0.778 \pm 0.137$	$0.0025 \pm 0.0021$
<b>SJ</b>	12	8	$0.894 \pm 0.078$	$0.0057 \pm 0.0038$
<b>TX</b>	12	3	$0.318 \pm 0.164$	$0.0021 \pm 0.0018$

closest to the major Gulf haplotype.

Parsimony and Maximum-likelihood failed to demonstrate reciprocal monophyly among Atlantic/Gulf lines (Fig. 2.5). Thus our phylogenetic analysis provides no signature of the historical vicariant event hypothesized to have shaped the patterns of genetic differentiation in other coastal marine species from the Southeastern United States.

### Discussion

On a local scale, populations of the dusky pipefish demonstrate significant genetic population structure due to a strong isolation by distance effect. On a regional scale, Bayesian microsatellite analysis supports a genetic break between Atlantic and Gulf of Mexico populations of *S. floridae*. This result is slightly incongruent with *mtDNA* analyses that fail to demonstrate reciprocal monophyly between Atlantic and Gulf populations. Moreover, high levels of gene flow from *mtDNA* analyses suggest that a barrier to gene flow between Atlantic and Gulf populations for this species is unsubstantiated. Thus, although population structuring is evident within the two geographic provinces, gene flow is not sufficiently restricted between Atlantic and Gulf of Mexico populations to justify the subspecies designations that were once in place for those particular populations of dusky pipefish. The inclusion of additional data likely would not change this outcome, because other phylogeographic studies of pipefish found well-supported reciprocal monophyly over smaller distances and with less sequence information (300bp) from the mitochondrial *cytochrome b* gene than was

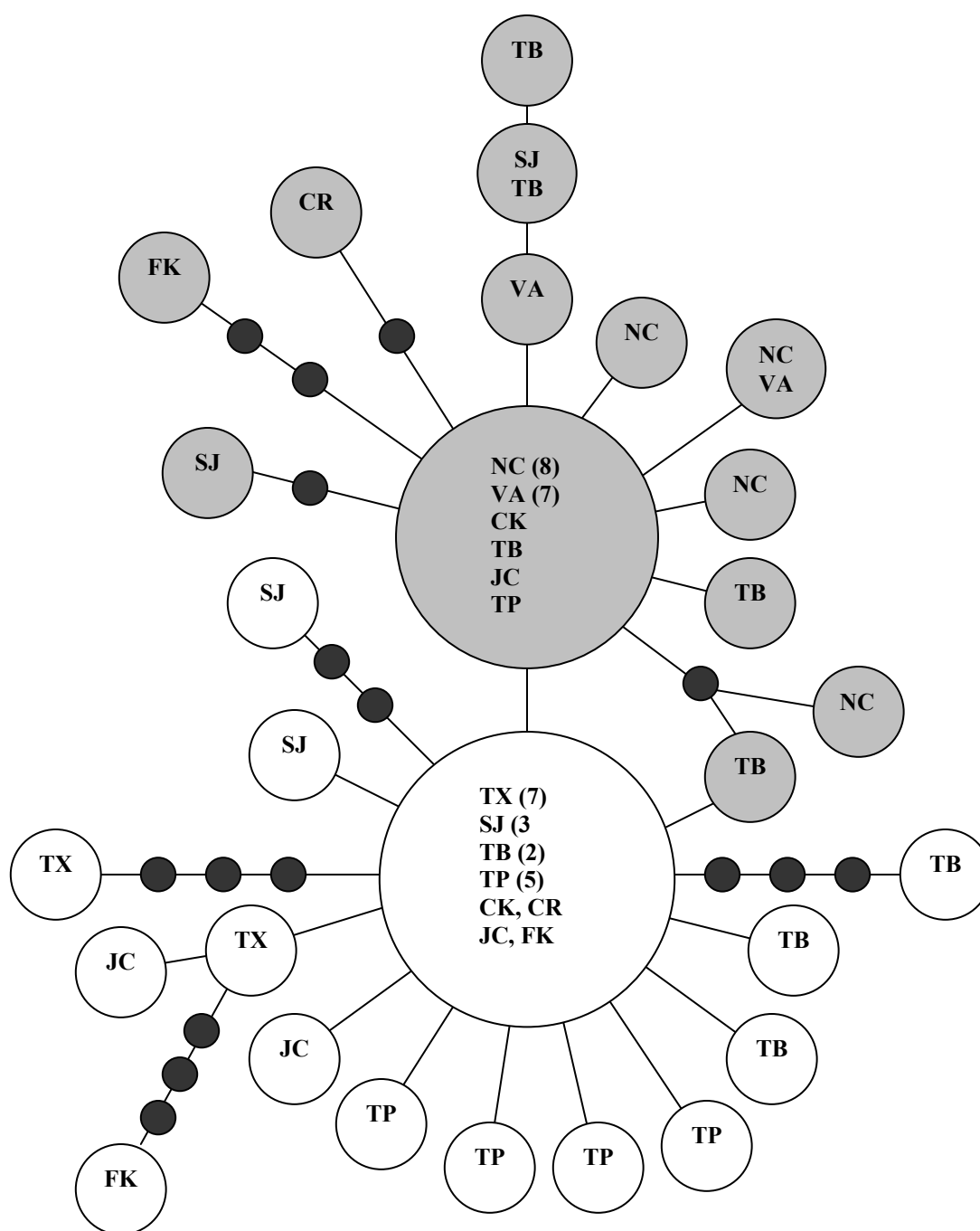
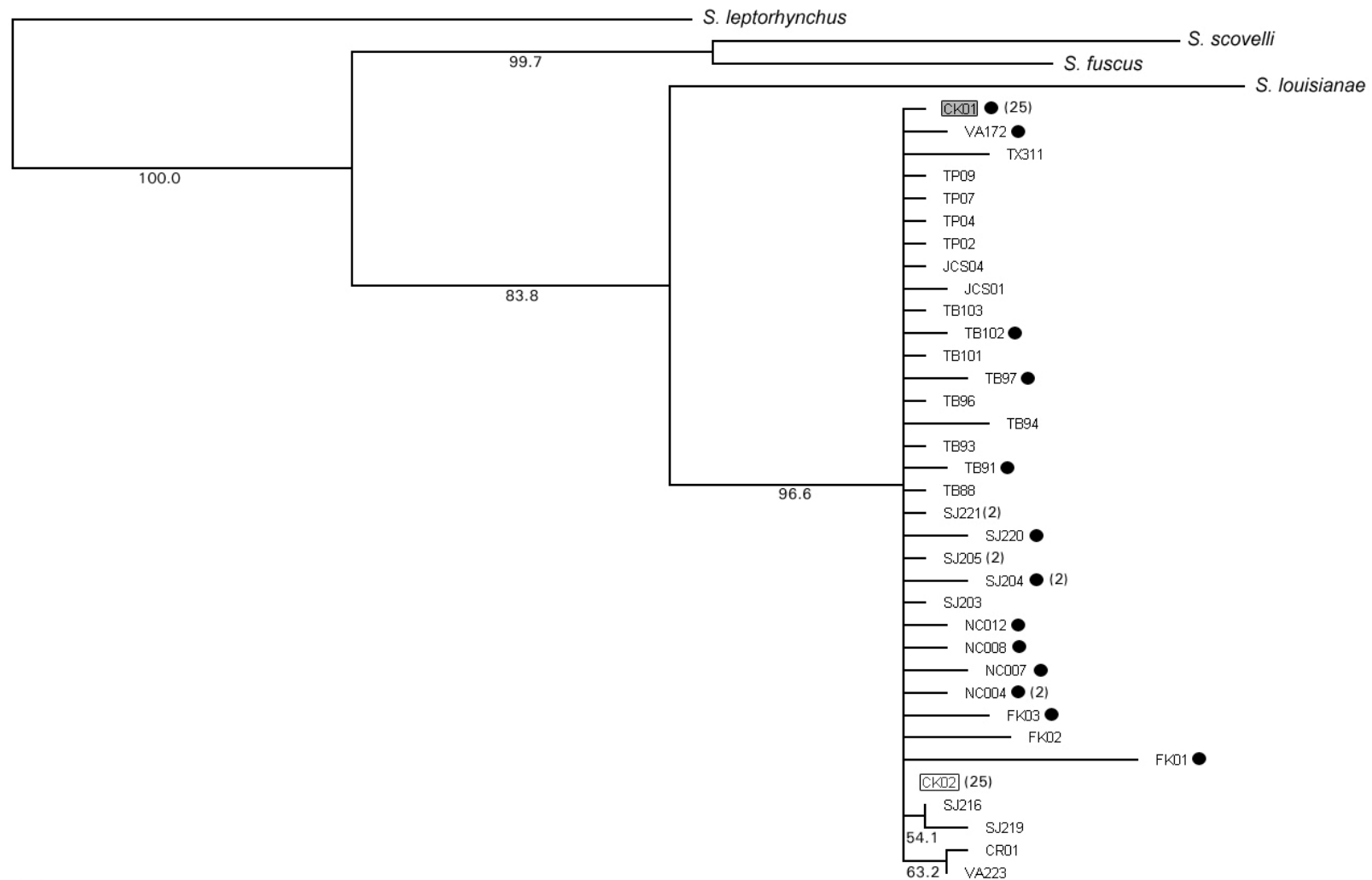


Figure 2.4. Statistical parsimony (95%) network for 28 mtDNA haplotypes identified in *S. floridae* samples. Each connection is a single mutational step and black circles represent inferred haplotypes. Numbers in parentheses indicate the frequency of the haplotype in that particular location; single occurrences of a haplotype do not have a number. Size of circles is proportional to the number of individuals with that haplotype. Grey-shaded haplotypes code for Thymidine at position 374 of *cytochrome b* fixed for all individuals sampled from the Atlantic sites.



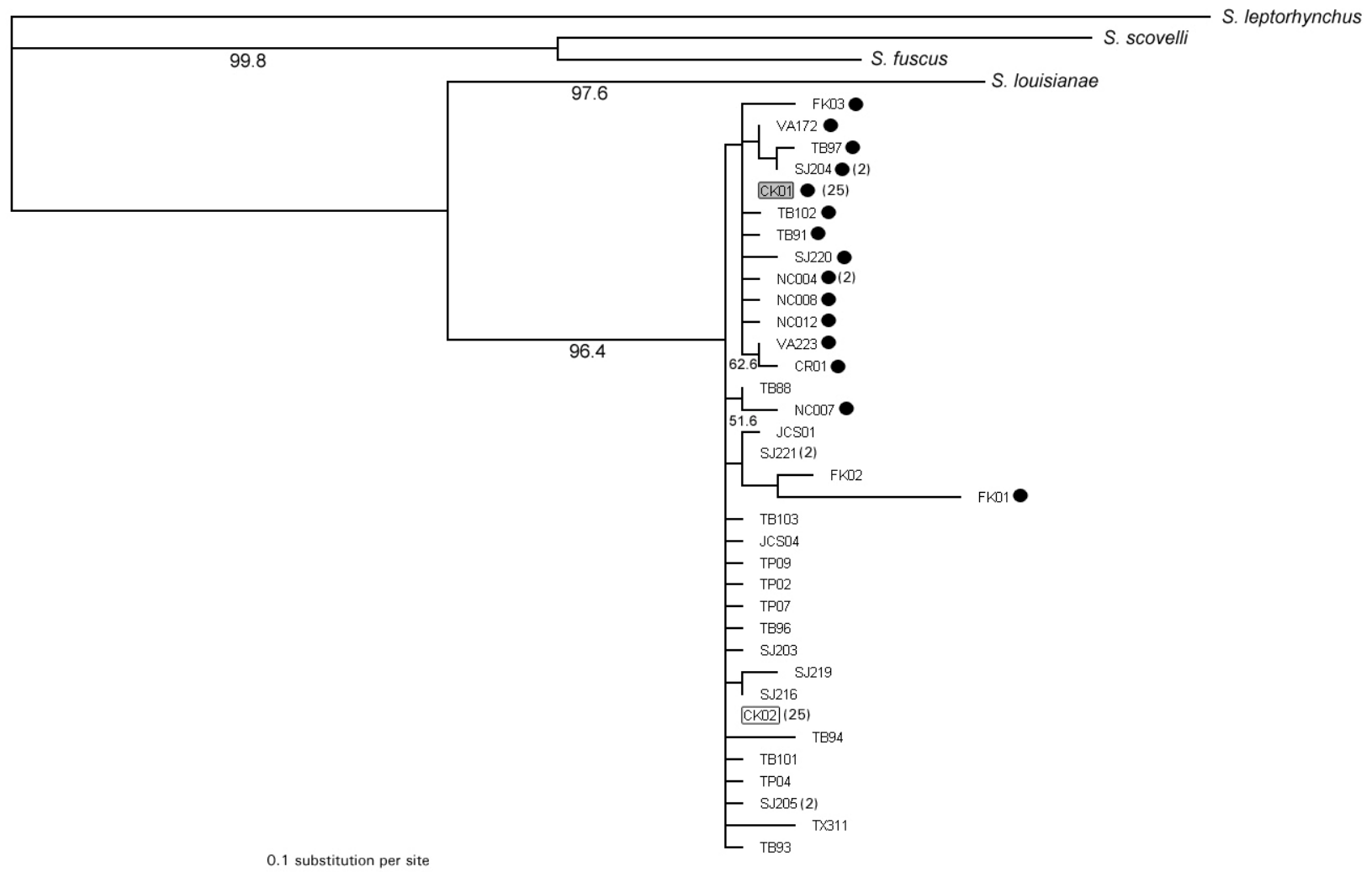


Fig 2.5 continued (b) Maximum Likelihood consensus tree.

employed in the present study (Chenoweth et al. 2002).

A strong isolation by distance pattern suggests this species is philopatric with limited migration and dispersal between populations and between ocean basins. All members of the family Syngnathidae have a mating behavior characterized by male parental care, and most species lack a free-living larval stage. Because syngnathids lack a larval stage and have poor swimming capabilities, dispersal of juveniles and adults likely is limited (Lourie & Vincent 2004). Other species of fish without a pelagic larval stage have shown strong genetic structuring between populations (Doherty et al. 1995, Hoffman et al. 2005). However, the dusky pipefish may differ from some of these other species, because this and other species of pipefish migrate to deeper waters during the winter months (Brown 1972, Mercer 1973, Lazzari & Able 1990). Individuals from distinct populations may mix during the winter, possibly even mating in the deeper water, or may return to different shallow seagrass meadows from one year to the next. Juveniles of this species are also often found in floating vegetation that may provide shelter to transport individuals several hundred kilometers in ocean currents (Dawson 1982). While this may explain the existence of this species in Bermuda (Dawson 1982), it is unlikely that migration of individuals from the Atlantic to the Gulf of Mexico takes place because of the strong northerly influence of the Gulf Stream Current on larval fish transport (Hare et al. 2002) and the dearth of suitable seagrass habitat along the eastern coast of Florida (Dawson 1982).

The result that Atlantic and Gulf of Mexico populations of dusky pipefish share genetic material across the Avise's biogeographical line in southeastern Florida, as



evidenced by mitochondrial data, is interesting given the widespread support for this barrier to gene flow in range of other coastal maritime taxa (Avice 2000). This phylogeographic pattern is far from universal however, and several species of fishes and at least one species of bivalve do not show the expected phylogenetic separations between the Atlantic and Gulf (Gold & Richardson 1998, Lee & Foighil 2005). It is interesting to note that coastal species that register little or no geographic divergence are species that undergo extensive migrations to and from adult breeding grounds (Avice et al. 1986, Gold & Richardson 1998) and/or species that have pelagic larvae that may be transported over several hundred kilometers (Gold & Richardson 1998, Lee & Foighil 2005). Given the diminutive potential for dispersal and adult migration in this species, this is an unlikely scenario to explain the apparent gene flow between populations of pipefish.

Other species of syngnathids have shown congruence with documented natural barriers to gene flow (Lourie & Vincent 2004). For example, the unique life history of this family may explain the distribution patterns of haplotype groupings of the three-spot seahorse, *Hippocampus trimaculatus*, on either side of Wallace's Biogeographic Line (Lourie & Vincent 2004). Other species of syngnathids have shown strong divergence in the absence of any known natural barrier to gene flow such as the hairy pipefish, *Urocampus carinirostris*, found on the west coast of Australia. This species shows two monophyletic groups that have a strong clinal intergradation signal over a small (130km) distance (Chenoweth et al. 2002). The authors attribute this biogeographic pattern to secondary contact owing to a recent population expansion from a small refuge

population during recent glaciation events (Chenoweth et al. 2002). Similarly a recent investigation in the population genetics of the bay pipefish, *Syngnathus leptorhynchus*, has documented a recent post-glaciation radiation into Alaskan waters from southern glacial refugia (Wilson et al. 2006). Interestingly, this species shows no reduction in gene flow across Point Conception, a natural boundary thought to limit gene flow in several marine species on the west coast of North America (Palumbi 1994, Wilson et al. 2006). Finally, at least one species of the seahorse shows a lack of mitochondrial structuring as estuarine populations share haplotypes over relatively small spatial scales (Teske et al. 2003).

The results of this study present a particular challenge to explain in the context of gene flow across Avise's biogeographic line. One plausible explanation is that these populations were recently isolated from one another and have not yet reached equilibrium with respect to genetic diversity in the east coast of Florida (Palumbi 1994). Another explanation is that these populations underwent secondary contact on the east coast of Florida recently. A third possibility is that a third population representing Caribbean and southern Atlantic populations may influence the genetic structure of the east coast of Florida. Two highly divergent sequences were obtained from the Florida Keys and may represent recent immigration from southern gene pools. At least one species of bivalve does not show a genetic disjunction across peninsular Florida due to the influx of genetic material from the Caribbean basin suggesting that pseudocongruence would explain the pattern of the biogeographic split if Caribbean samples were not entered into analysis (Lee & Foighil 2005). Although these

explanations are beyond the scope of this investigation, future studies that incorporate additional samples throughout the dusky pipefish's range may shed light on the patterns of phylogeography within this species.

**CHAPTER III**

**GEOGRAPHIC VARIATION IN THE GENETIC MATING SYSTEM OF THE**

**DUSKY PIPEFISH, *SYNGNATHUS FLORIDAE*.**

**II: ATLANTIC VERSUS GULF OF MEXICO CASE STUDY\***

**Introduction**

Sexual selection can lead to rapid evolution of behavioral and morphological traits, and thus may be a salient force in species divergence (Kraaijeveld & Pomiankowski 2004). The recent flood of molecular appraisals of mating behavior has established that the genetic mating system is closely related to the intensity of sexual selection in nature (Jones et al 2001a; Shuster & Wade 2003). Consequently, it is now clear that knowledge of the mating system is fundamental to a complete understanding of sexual selection in any system. Data on mating systems from a wide variety of species indicate that mating behavior can be evolutionarily labile with important evolutionary consequences (Kusmierski et al. 1997, Petrie & Kempenaers 1998; Griffith 2000; Avise et al. 2002). However, genetic characterizations of the mating system for most species involve only a single exemplar population, despite the fact that mating systems likely vary over space and time (Jones et al. 2001b). Such geographic or temporal variation in mating systems would provide excellent opportunities for comparative studies of mating system evolution. For example, understanding the causes of mating system variation among populations could resolve why some lineages

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experience intense sexual selection whereas others do not (Panhuis et al. 2001).

Arguably, this question is one of the central issues in the study of sexual selection.

Mating system divergence among populations also theoretically facilitates speciation

(Kraaijeveld & Pomiankowski 2004). Lande (1982) showed that sexual selection has the

potential to initiate sexual isolation and character divergence as distinct populations

diverge with respect to secondary sexual traits and mating preferences. Such divergence

is most effective when populations evolve dissimilar mating systems. In addition, Zeh &

Zeh (2000) have speculated that mating system divergence could facilitate speciation

through parent-offspring conflict. Despite the apparent potential of studies of

geographically or temporally varying mating systems, such studies are thus far rare.

Several factors have slowed progress in the comparative study of mating systems

within species. An understanding of evolutionary processes such as sexual selection

requires the knowledge of biological parentage at a level of detail that in most species

can be achieved only through the application of molecular methods (Avisé 2004). Such

studies are expensive and time consuming. A comprehensive study of the mating system

in a single population may involve hundreds or thousands of genotypes (Avisé et al.

2002), so scaling up to multiple populations can be a major logistical problem. Mating

system studies also require samples of breeding adults and their offspring, which can be

difficult to obtain from multiple populations across the range of a species, especially for

species whose abundance varies temporally and geographically. As a consequence of

these limitations, studies of sexual selection often are forced to assume that mating

systems are fixed or nearly fixed over time and space within a species. For many species

this assumption may be false since populations that inhabit distinct environments may experience local environmental selection pressures, such as predation, that can influence the genetic mating system (Kelly et al. 1999, Bronikowski et al. 2002). In addition to geographic variation in mating systems, populations can be temporally dynamic with respect to population density, the availability of mates, and the breeding condition of individuals, all of which may affect the mating system (Emlen & Oring 1977, Shuster & Wade 2003, Soucy & Travis 2003). Thus, to gain a more complete understanding of the ecological factors that influence the evolution of genetic mating systems, a deeper appreciation of within-species mating system dynamics is necessary.

The few studies that have addressed geographic variation in mating systems have produced mixed results, ranging from significant intraspecific variation among populations of fishes (Trexler et al. 1997, Kelly et al. 1999, Soucy & Travis 2003), birds (Griffith et al. 1999, Griffith 2000, Durrant & Hughes 2005) and mammals (Taylor et al. 2000, Clinchy et al. 2004) to essentially no variation between geographically distinct populations (Zane et al. 1999, Jones et al. 2001b, Goodisman et al. 2002). A handful of comparative studies have attempted to correlate variation in mating patterns with environmental factors. While these studies have also led to mixed results (Weatherhead & Boag 1997, Griffith et al. 1999), some biologically important patterns have emerged. First, in birds, extra-pair fertilizations are more frequent in mainland populations than in island populations (Griffith et al. 1999, Griffith 2000, Griffith et al. 2002). Second, in Trinidadian guppies the frequency of multiple insemination is related to the intensity of predation (Kelly et al. 1999, Bronikowski et al. 2002). Finally, in a study of three

populations of least killifish, rates of multiple paternity were higher in populations with greater population densities (Soucy & Travis 2003). These studies provide an interesting preliminary glimpse into environmental factors that likely shape mating systems, and hence sexual selection, in nature, but are of insufficient number and taxonomic breadth to provide any clear generalities. Additional studies of geographic variation in mating systems are warranted.

In this study we characterize the genetic mating system of two geographically distinct populations of the dusky pipefish, *Syngnathus floridae*. The reproduction of this species is characterized by male pregnancy in which females transfer eggs into a specialized brood pouch on the male's trunk during copulation (Dawson 1982; Jones & Avise 2003). Males then provide parental care during development until the young are released as independent juveniles (Dawson 1982). This species is likely sex-role reversed with respect to the intensity of sexual selection, such that sexual selection acts more strongly on females than on males (Jones & Avise 1997b). The high paternal investment in this and other syngnathid species can depress the potential reproductive rates of males to such an extent that the direction of sexual selection becomes reversed relative to that of most taxa (Berglund et al. 1989, Jones et al. 2005). Characterization of the mating system is facilitated in this species because maternal genotypes can be reconstructed from genetic analysis of pipefish embryos contained within the male brood pouch through the use of highly polymorphic molecular markers (Jones & Avise 1997b). The dusky pipefish is ideally suited for this type of inquiry because it has a wide geographic distribution and large numbers of pipefish can be harvested from relatively

small areas during the breeding season, yielding high numbers of pregnant males and their potential mates.

The study of geographic variation in the mating system of the dusky pipefish provides a good opportunity to take advantage of quantitative measures of the potential for sexual selection based on Bateman's principles. Even though these techniques have been advocated as methods for the quantification and comparison of mating systems (e.g. Arnold 1994, Arnold & Duvall 1994, Jones et al. 2001a), they have not yet been employed to compare mating systems among populations of the same species. One major advantage to the application of Bateman's principles for the characterization of mating systems in an inter-population framework is that it allows strict quantitative comparison of the potential for sexual selection between populations and between sexes.

The present study has several goals. First, we use Bateman's principles to test for significant geographic variation in the mating systems of two geographically isolated populations of dusky pipefish. Second, we examine temporal variation in the mating system by comparing two collections made in 2003 within one Florida population. Third, we consider the results in light of differences in population density and other environmental factors that may influence the genetic mating system.



## Materials and Methods

### *Study species*

The dusky pipefish, *S. floridae*, is a geographically widespread species, occurring in the Western Atlantic Ocean from the Chesapeake Bay and Bermuda to the Southern Florida coast and the Florida Keys as well as in the Gulf of Mexico from the West coast of Florida to Panama (Dawson 1982). This species inhabits shallow seagrass beds in coastal waters, and its diet comprises primarily zooplankton such as copepods, amphipods, isopods and mysids (Brown 1972, Mercer 1973). During mating, females deposit eggs in a highly specialized brood pouch on the male's ventral surface. The male then fertilizes the eggs and carries the embryos until they hatch approximately 10 days later (Dawson 1982). In the Chesapeake Bay, near the northern limit of its range, *S. floridae* migrates inshore during the spring and breeds from April to October with peaks in population density and male pregnancy in July and August (Mercer 1973). In the Northern Gulf of Mexico, populations of *S. floridae* mate from April through November with a peak in population density and male pregnancy in August and September (Brown 1972).

### *Collection of specimens*

We collected 150 adult male and 91 adult female *S. floridae* on 5 and 6 August 2003 from Mobjack Bay (N37°18.423', W76°24.752'), near the mouth of the York River, Virginia (Table 3.1). Two samples of *S. floridae* were collected from St. Joseph Bay, Florida (N29°47.765', W85°18.237') on 18-20 July 2003 (19 males, 34 females)

and 24-25 August 2003 (17 males, 30 females) near the collection site reported in Jones & Avise (1997b). Individuals were captured at each location by seine net (2 mm mesh) from measured plots marked with stakes inside a shallow (depth less than 1m), continuous seagrass meadow. Our goal was to capture as many pregnant males as possible along with their potential mates within the designated area. Each plot was seined completely during low tide a minimum of three full sweeps or until a full sweep of the area captured less than five percent of the original sweep. We measured standard lengths (SL, tip of snout to base of caudal fin) of all individuals. All fishes were sacrificed in the field by severing their spinal column above the operculum and preserved in 95% EtOH.

Males were considered sexually mature if they possessed a mature brood pouch, whereas females were considered sexually mature if they possessed ripe ova. All females of less than 120mm standard length were dissected to assess the presence of ripe ova, whereas females longer than 120mm were assumed to be sexually mature (Brown 1972). This assumption was confirmed by random dissections on 15 females >120mm from each population, all of which contained ripe ova. Based on these criteria all individuals collected from these sites were sexually mature adults and were included in all analyses. The adult sex ratio (ASR) is given as the number of adult males divided by the total number of adults collected, whereas the operational sex ratio (OSR) is the ratio of receptive adult males to the total number of receptive adults. In the case of sex-role-reversed pipefish, such as *S. floridae*, the OSR is the number of non-pregnant males

Table 3.1. Comparison of sample sizes of adults ( $n$ ), males ( $m$ ), non-pregnant males ( $m'$ ) and females ( $f$ ), adult sex ratios (ASR; ratio of males to total adults), operational sex ratios (OSR; ratio of non-pregnant males to females + non-pregnant males), population density (ind/m<sup>2</sup>) and mean adult female population size (estimated using the modified Lincoln-Peterson method with 95% confidence intervals) of *Syngnathus floridae* from different sample sites.

Site	Date	Sample Size				ASR	OSR	Population Density (ind/m <sup>2</sup> )			Adult Female Population Size (95% CI)
		$n$	$m$	$m'$	$f$			Total	$m$	$f$	
Mobjack Bay, VA	8/03	241	150	3	91	0.62	0.03	0.18	0.11	0.07	635 (323-947)
St. Joseph Bay, FL	7/03	53	19	6	34	0.36	0.15	0.06	0.02	0.04	122 (52-191)
	8/03	47	17	6	30	0.36	0.17	0.03	0.01	0.02	162 (42 – 282)
	7/94 <sup>1</sup>	93	50	7	43	0.54	0.14	---	---	---	138 (85 – 192)

<sup>1</sup>Data for the June 1994 St. Joseph Bay collection from Jones & Avise (1997b)

divided by the sum of non-pregnant males and adult females (Kvarnemo & Ahnesjö 1996).

#### *Molecular parentage analysis*

Parentage analysis was conducted on the broods of 30 pregnant males from the Virginia population and 11 pregnant males from each of the Florida collections. Brood pouches were dissected following the protocol of Jones & Avise (1997b). Briefly, each brood pouch was divided into 14 roughly equal sections (once lengthwise and six times laterally) and marked with an alcohol resistant pen. Three embryos were selected at random from each section, extracted with flame-sterilized forceps and placed into a 96-well plate for genotyping. Using this sampling scheme, we genotyped a total of 42 offspring from each male's brood pouch and counted the total number of embryos in each section of the pouch.

We used three polymorphic microsatellite loci to characterize the mating system of *S. floridae* (*Micro11.1*, *Micro22.3* and *Micro25.22*) and four polymorphic microsatellite loci to genotype all sexually mature adults in each population (*Micro11.1*, *Micro22.3*, *Micro25.10* and *Micro25.22*, Table 3.2). Microsatellite markers employed in this study were originally developed for the Gulf pipefish, *S. scovelli* (Jones & Avise 1997a; Jones et al. 1999). DNA was extracted using a standard Proteinase K and 5% Chelex digestion in 96-well plates (Miller & Kapuscinski 1996), and microsatellite markers were amplified using polymerase chain reaction (PCR) conditions modified from Jones & Avise (1997a). We used annealing temperatures of 54°C for *Micro11.1*

Table 3.2. Microsatellite data comparing the number of alleles ( $A$ ), sample sizes ( $N$ ), observed and expected heterozygosities ( $H_O$  and  $H_E$  respectively) and  $F_{ST}$  estimates between Virginia and pooled Florida adult *Syngnathus floridae* collections.

<i>Locus</i>	Mobjack Bay, VA				St. Joseph Bay, FL				<i>A</i>	$F_{ST}$
	<i>A</i>	<i>N</i>	$H_O$	$H_E$	<i>A</i>	<i>N</i>	$H_O$	$H_E$		
<i>Micro11.1</i>	43	228	0.820	0.855	47	99	0.950	0.960	65	0.076
<i>Micro22.3</i>	21	191	0.859	0.800	22	68	0.882	0.927	27	0.101
<i>Micro25.10</i>	18	185	0.838	0.871	21	71	0.859	0.886	25	0.007
<i>Micro25.22</i>	20	228	0.842	0.873	22	98	0.918	0.916	25	0.015
<i>Global <math>F_{ST}</math></i>										<b>0.051</b>

and *Micro22.3*, and 56°C for *Micro25.10* and *Micro25.22*. All thermal cycling ran for 36 cycles, except that for *Micro11.1*, which ran for 40 cycles to yield sufficient PCR product for analysis. Primers were labeled with a fluorescent dye and sized on an ABI 3730 Genetic Analyzer (Applied Biosystems, Foster City, CA). All microsatellite fragment analysis was accomplished using Genotyper<sup>®</sup> or Genemapper<sup>®</sup> software (Applied Biosystems, Foster City, CA).

Maternal genotypes were reconstructed from progeny arrays using GERUD2.0 (Jones 2001, 2005), and the resulting inferred maternal genotypes were compared with each other and with the genotypes of collected adult females in the population using Microsatellite Toolkit 3.1 for Microsoft Excel (Park 2001). Because eggs are spatially segregated by maternity in male brood pouches (Jones & Avise 1997b), we were able to assign embryos to mothers with great accuracy based on data from the genotyped offspring in each of the 14 demarcated sections. If only one maternal genotype was found in all three embryos for a particular section, all embryos counted in that section were ascribed to that inferred female. When a section transitioned from one mother to the next, we assigned one third and two thirds of the total embryos to each of the two mothers, depending upon the proportion of genotyped embryos genetically assigned to each mother.

Female reproductive contribution was calculated by tallying all embryos assigned to each mother for all sections. Eggs that had no visible signs of embryonic development were considered undeveloped eggs. These eggs were likely the result of unsuccessful fertilization or incomplete development. Because undeveloped eggs

contribute a small proportion to the numbers of eggs received by males ( $2.4\% \pm 1.5 SE$  for the Virginia population and  $8.4\% \pm 1.7 SE$  for July and August Florida populations) and because it is unlikely that these eggs would result in viable offspring, undeveloped eggs were not included in the female reproductive contribution or estimates of male reproductive success.

All microsatellite data were analyzed with GENEPOPv3.4 (Raymond & Rousset 1995) to test for Hardy-Weinberg equilibrium (Fisher's exact test) and for genotypic disequilibrium for pairs of loci within the population (Fisher's exact test). Wright's F-statistic ( $F_{ST}$ ) was also calculated using GENEPOPv3.4 (Table 3.2). The cumulative probability of identity ( $P_{ID}$ ) was estimated from microsatellite data for adult females and reconstructed female genotypes from the two different populations using LOCUSEATERv2.4 (Hoyle et al. 2005). A modified Lincoln-Peterson method of capture-recapture was used to estimate female local population size in the Virginia and Florida populations based on number of reconstructed female genotypes (Jones & Avise 1997b).

#### *Quantification of the genetic mating system by Bateman's principles*

The genetic mating system for males from each geographic location was quantified using mean mating success ( $\bar{X}_{ms}$ ), mean reproductive success ( $\bar{X}_{rs}$ ) and Bateman's three principles: the opportunity for selection ( $I$ ), the opportunity for sexual selection ( $I_s$ ), and the sexual selection gradient or Bateman gradient ( $\beta_{ss}$ ). Mean mating success is the average number of mates per male for each population. Similarly,  $\bar{X}_{rs}$  is the mean number of offspring per male for each population. The opportunity for

selection is the standardized variance in reproductive success [ $I = \sigma_{rs}^2 / \bar{X}_{rs}^2$ ], and the opportunity for sexual selection is the standard variance in mating success [ $I_s = \sigma_{ms}^2 / \bar{X}_{ms}^2$ ] (Arnold 1994, Arnold & Duvall 1994). The Bateman gradient is the slope of the weighted least-squares regression of relative reproductive success (number of offspring divided by the mean) on mating success (Arnold 1994, Jones et al. 2002). All measures of the genetic mating system include an estimate of non-breeding males (Wade 1979, Arnold 1994, Shuster & Wade 2003). The number of non-breeding males was calculated based on the frequency of non-pregnant males encountered at each location and during each sampling period.

### *Statistical analysis*

Statistical analyses were performed with JMP IN™ v5.1 statistical software package (SAS Institute Inc., Cary NC). All statistics were analyzed first for normality and equal variances. If these assumptions were not met, data were transformed or if no transformation satisfied *a priori* assumptions, appropriate non-parametric tests were applied. Statistical tests as well as any transformations are indicated throughout the text. All means are reported  $\pm$  one standard error of the mean (SE).

## **Results**

### *Microsatellite markers*

Four microsatellite loci revealed high levels of polymorphism and heterozygosity among adult *S. floridae* in both populations (Table 3.2). The Virginia population



displayed between 18 and 43 alleles per locus with expected heterozygosities ranging from 0.80 to 0.87 (Table 2). The Florida sample displayed between 21 and 47 alleles per locus with expected heterozygosities ranging from 0.89 to 0.96 (Table 2). Fisher's exact tests indicated no significant departures ( $\alpha = 0.05$ ) from Hardy-Weinberg equilibrium or linkage equilibrium. A low-frequency (0.002) null allele was detected at *Micro11.1* in the maternal parent of the brood of a single male from the Virginia population. The allele was discovered after GERUD2.0 (Jones 2001, Jones 2005) indicated the presence of two maternal genotypes in the male's offspring that were inconsistent with the expected spatial clustering of offspring by maternity. The null allele manifested itself clearly as sets of embryos homozygous for each paternal allele with an absence of embryos possessing the expected heterozygous genotype comprising both paternal alleles. A second null allele at *Micro11.1* was discovered in the maternal contribution to the broods of two Florida males that apparently had mated with the same female, as evidenced by identical reconstructed maternal genotypes. These null alleles were infrequent enough in both populations that their presence did not cause a heterozygosity deficit (Fisher's exact test,  $P < 0.05$ ), and they did not compromise the interpretation of the parentage analysis. Moderate population differentiation was evident from the  $F_{ST}$  values estimated for these four microsatellite markers (Table 2). Single locus  $F_{ST}$  estimates ranged from 0.007 – 0.101 with a global  $F_{ST}$  value of 0.051.

*Comparison between Virginia and Florida populations*

The Florida and Virginia sites differed in both ASR and OSR estimates. The ASR of the Virginia population caught in August 2003 was heavily male biased with a significant departure from an equal sex ratio ( $\chi^2 = 14.4$ , d.f. = 1,  $P < 0.0001$ , Table 3.1). The ASRs of the Florida collections were similar to one another and showed a significant female bias in the July ( $\chi^2 = 9.3$ , d.f. = 1,  $P = 0.002$ ) but not the August sample ( $\chi^2 = 3.6$ , d.f. = 1,  $P = 0.058$ ; Table 3.1). In contrast, OSRs were heavily female biased in all of the populations due to the rarity of non-pregnant males (Table 3.1). The two Florida collections showed similar OSRs, both of which were less female biased than the OSR in the Virginia population, suggesting that competition for males may be more intense in the latter population.

Males and females were significantly larger in body size (as measured by SL) in both Florida collections than in the Virginia population as shown by an two-way analysis of variance (ANOVA) and a Tukey-Kramer post-hoc analysis (male ANOVA:  $F_{2,184} = 43.6$ ,  $P < 0.0001$ , female ANOVA:  $F_{2,153} = 30.8$ ,  $P < 0.0001$ ). Some evidence of sexual dimorphism is present in the Virginia collection as females were slightly, but significantly, larger than males (male SL =  $128 \pm 1$  mm, female SL =  $133 \pm 2$  mm, ANOVA:  $F_{1,238} = 5.94$ ,  $P = 0.016$ ). No evidence of sexual dimorphism was present in the Florida samples as ANOVA detected no significant difference in SL between sexes ( $F_{1,96} = 0.0050$ ,  $P = 0.94$ ). However a significant difference in body size between the two Florida collections was observed (ANOVA:  $F_{1,96} = 7.37$ ,  $P = 0.008$ ). Adult *S. floridae* were, on average, 8 mm larger in the August population (July SL =  $148 \pm 2$  mm;

August SL =  $156 \pm 2$  mm). No significant interaction between time and sex was observed between Florida populations (ANOVA:  $F_{1,96} = 0.0095$ ,  $P = 0.92$ ).

### *Male mating behavior*

An evaluation of the July and August Florida collections revealed similar mating system estimates. The July population had six unmated males, one singly mated male, seven males that mated with two females, and three males that mated with three females ( $\bar{X}_{ms} = 1.5 \pm 0.3$ ). The August population had six unmated males, five singly mated males, four males that mated with two females, one triply mated male, and one male that mated with four females ( $\bar{X}_{ms} = 1.2 \pm 0.3$ ). Males from the two Florida collections also contained similar numbers of embryos, with an average of  $182.0 \pm 39.3$  embryos per male in July and  $177.6 \pm 38.2$  in August. Because the mating frequency ( $\chi^2 = 6.17$ , d.f. = 4,  $P = 0.19$ ), mean reproductive success (ANOVA:  $F_{1,31} = 0.0089$ ,  $P = 0.93$ ), mean mating success (ANOVA:  $F_{1,31} = 0.63$ ,  $P = 0.43$ ) and the variance in mean mating success (Levene's homogeneity of variances test:  $F_{1,31} = 0.095$ ,  $P = 0.76$ ) were not significantly different between males in the July and August Florida collections, the two collections were pooled together to draw a more robust comparison against the Virginia population.

Males in Virginia had higher rates of mating and higher numbers of viable embryos per male than the Florida population. Among the 30 males analyzed, only two males had broods consisting entirely of full siblings. Of the remaining 28 males, 11 had two mates, 14 had three mates, three had four mates and three were unmated ( $\bar{X}_{ms} = 2.5$

Table 3.3. Comparison of the opportunity for selection,  $I$ , the opportunity for sexual selection,  $I_s$ , and the Bateman gradient ( $\beta_{ss} \pm SE$ ) between Florida and Virginia populations of male *Syngnathus floridae*. Average reproductive ( $\bar{X}_{rs}$ ) and mating ( $\bar{X}_{ms}$ ) successes per male and their respective variances ( $\sigma^2$ ), including unmated males, are also listed.

Site	Date	Reproductive success			Mating success			Bateman gradient
		$\bar{X}_{rs}$	$\sigma^2_{rs}$	$I$	$\bar{X}_{ms}$	$\sigma^2_{ms}$	$I_s$	$\beta_{ss}(\pm SE)$
Mobjack Bay, VA	8/03	251.3	9165.8	0.15	2.52	0.79	0.13	$0.25 \pm 0.07$
St. Joseph Bay, FL	7-8/03	180.2	23911.8	0.74	1.33	1.35	0.76	$0.59 \pm 0.08$
	7/94 <sup>1</sup>	440.4	60837.4	0.31	1.58	0.81	0.33	$0.50 \pm 0.09$

<sup>1</sup>Data for the June 1994 St. Joseph Bay collection from Jones & Avise (1997b)

$\pm 0.2$ , Table 3.3). A chi square test showed a significant difference in the distributions of mating success between the Florida and Virginia populations ( $\chi^2 = 13.1$ ; d.f. = 4,  $P = 0.011$ ; Fig. 3.1). Virginia males had, on average, a mean reproductive success of  $251.3 \pm 95.2$ , a value significantly higher than the pooled Florida estimate of  $180.2 \pm 26.91$  (ANOVA:  $F_{1,62} = 4.82$ ,  $P = 0.032$ ). The lower mean estimate of reproductive success for Florida males is driven primarily by the relatively high frequency of unmated males in the Florida population, despite similar values for the number of viable embryos per mated male in both Florida and Virginia collections (FL =  $270.2 \pm 20.0$ , VA =  $259.7 \pm 17.1$ ; ANOVA:  $F_{1,52} = 0.16$ ,  $P = 0.69$ ).

An analysis of covariance (ANCOVA) revealed mean mating success was positively correlated with SL for males in both populations ( $F_{1,49} = 26.90$ ,  $P < 0.0001$ ; Fig. 3.2). Similarly, the relationship between number of embryos and SL was significantly positive in both populations (ANCOVA:  $F_{1,40} = 7.14$ ,  $P = 0.01$ ), and mated Virginia males were significantly smaller than their Florida counterparts (ANCOVA:  $F_{1,49} = 23.81$ ,  $P < 0.0001$ ; Fig. 3.3).

Estimates of both  $I$  and  $I_s$  were significantly higher in the Florida than in the Virginia population (Levene's homogeneity of variances test:  $F_{1,64} = 33.04$ ,  $P < 0.0001$ ;  $F_{1,64} = 25.15$ ,  $P < 0.0001$ , respectively; Table 3.3). An analysis of covariance revealed that Florida males have a significantly steeper estimate of  $\beta_{ss}$  than Virginia males ( $F_{3,64} = 9.32$ ,  $P = 0.003$ , Table 3.3, Fig. 3.4). Both the Virginia and Florida collections exhibited a significant positive relationship between relative fitness and mating success, resulting

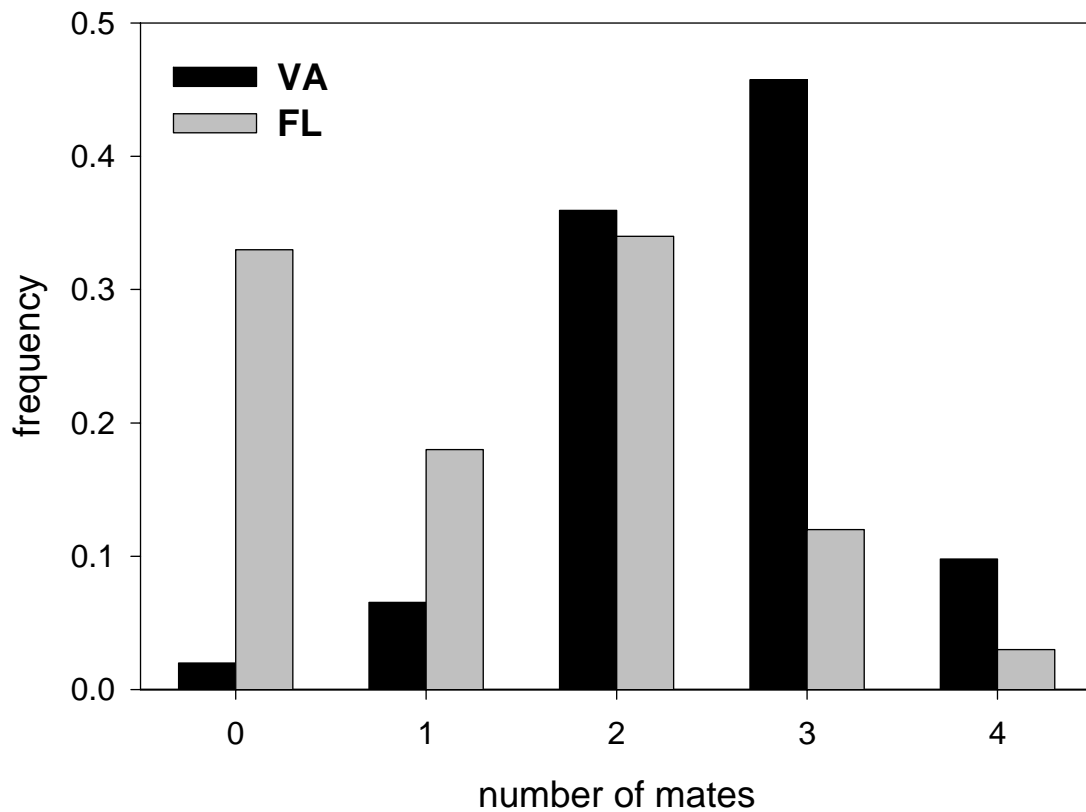


Figure 3.1. Frequency histogram of male mating success comparing Virginia (VA) and pooled Florida (FL) populations. The histogram is derived from genetic analysis of offspring from male brood pouches in the VA and FL collection, coupled with the consideration that 12 of the 36 males collected in Florida and 3 of the 150 males from Virginia had not mated. Distributions of mated individuals were significantly different between populations ( $\chi^2 = 13.120$ , d.f. = 4,  $P = 0.0107$ ).

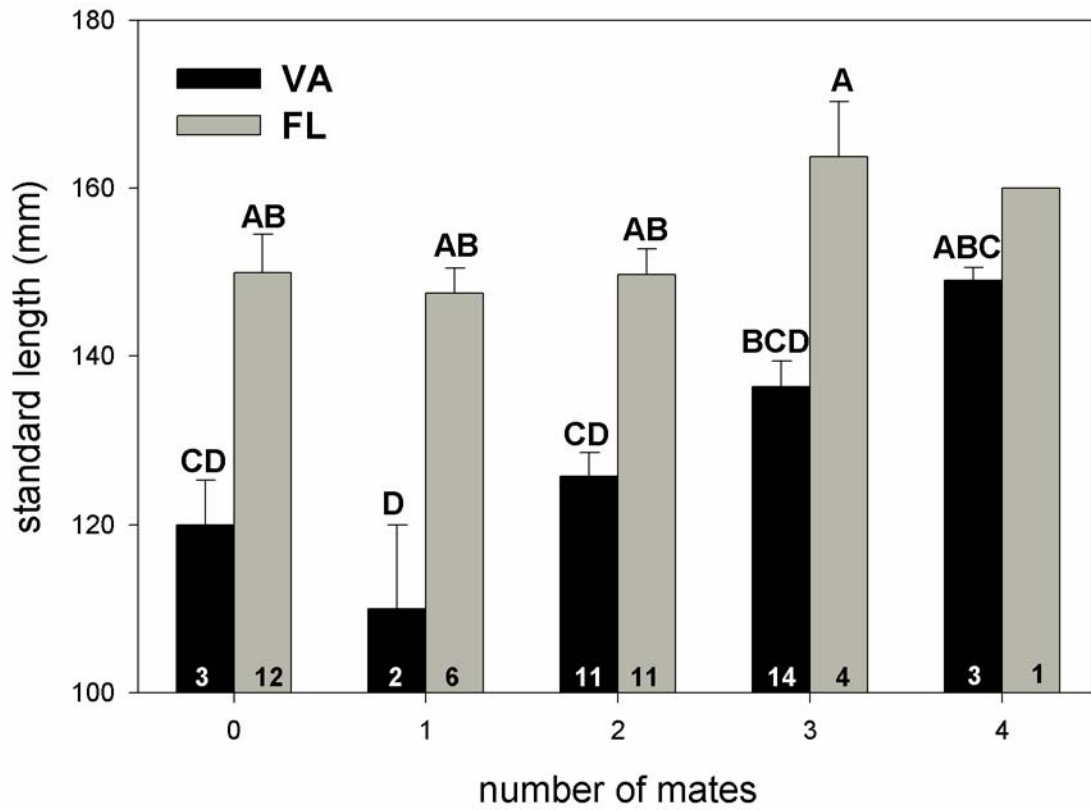


Figure 3.2. Mean standard length ( $\pm$ SE) of dusky pipefish males showing the increase in body size with increasing numbers of mates from the Virginia (VA) and pooled Florida (FL) collections. Numbers at the base of each bar represent the sample size. Bars with like letters have means that were not significantly different from one another (Tukey-Kramer HSD Test,  $\alpha = 0.05$ ).

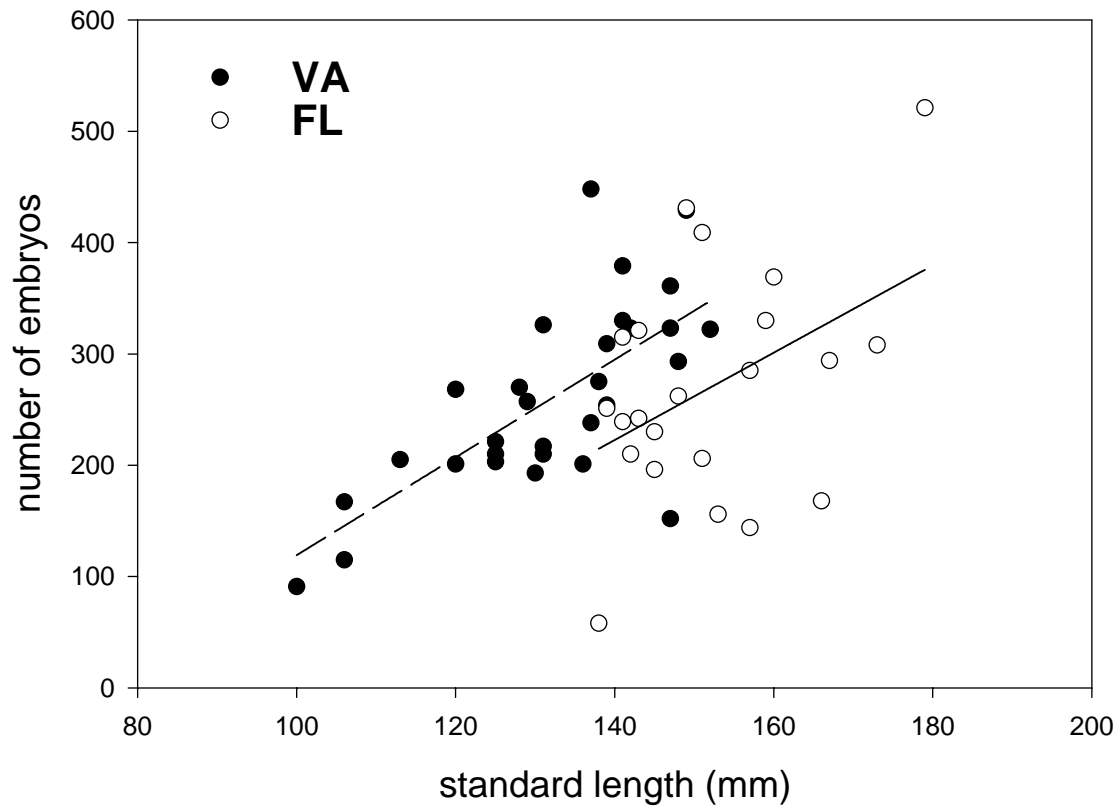


Figure 3.3. Relationship between numbers of embryos within the brood pouch and the standard length of mated male *Syngnathus floridae* from the Virginia (VA) and pooled Florida (FL) collections. Regression slopes of pooled Florida (solid,  $R^2 = 0.50$ ) and Virginia (dashed,  $R^2 = 0.18$ ) populations are shown. There is a significant positive relationship between number of embryos and male standard length for both populations (ANCOVA:  $F_{1,49} = 7.14$ ,  $P = 0.01$ ). Males from Virginia have significantly more offspring for a given body size than males from Florida (ANCOVA:  $F_{1,49} = 23.8$ ,  $P < 0.0001$ ).



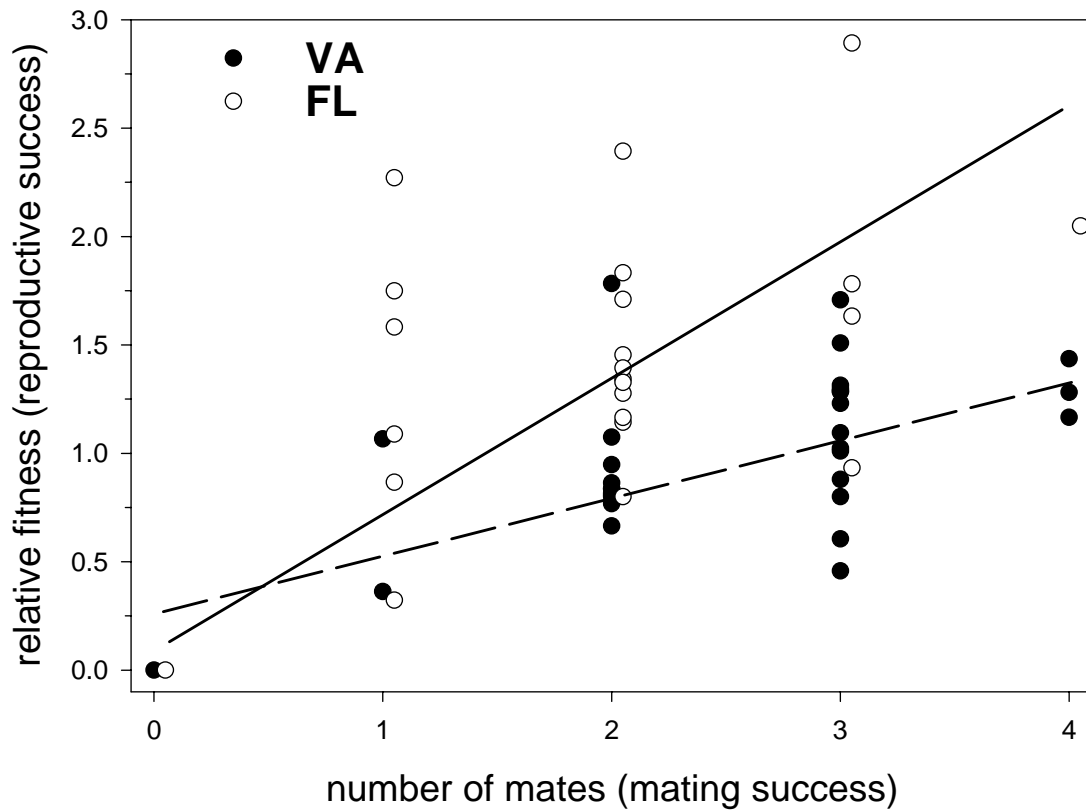


Figure 3.4. Relationship between reproductive success and mating success for male *Syngnathus floridae* from Virginia (VA) and Florida (FL) populations. Reproductive success is shown as relative fitness, i.e., number of offspring produced divided by the mean number of offspring produced. The sexual selection (Bateman) gradient is shown as the weighted least-squares regression line for FL (solid line) and VA (dashed line). The slopes of both the FL and VA Bateman gradients are significantly greater than zero (FL,  $P < 0.0001$ ; VA,  $P = 0.0007$ ).

in slopes of Bateman gradients significantly greater than zero (VA  $P = 0.0007$ ; FL  $P < 0.0001$ , Fig. 3.4).

### *Female mating behavior*

The high variability of the microsatellite markers allowed reconstruction of female genotypes, which we then compared to the genotypes of individual females caught in the field. We reconstructed 77 female genotypes from the Virginia population, 24 from the July Florida collection and 20 from the August Florida collection. The average probability of identity for collected adult females (FL =  $8.6 \times 10^{-7}$ , VA =  $8.1 \times 10^{-5}$ ) and reconstructed female genotypes (FL =  $3.6 \times 10^{-7}$ , VA =  $3.5 \times 10^{-5}$ ) were low, suggesting that a match between a reconstructed genotype and a female would only occur if we had collected the true mother of a progeny array. Ten adult females in the Virginia population shared identical three-locus genotypes with particular inferred mates of sampled males. Although not present in collected females, two identical reconstructed female genotypes were detected in more than one male's brood from the Virginia population, indicating that females have the potential to mate with more than one male. The July Florida population yielded five reconstructed maternal genotypes that matched females caught in the field. Two of these reconstructed genotypes were each recovered in the embryos of two distinct males (i.e., each of two females had deposited eggs in two of our sampled males). In addition to these two collected females that mated with at least two males, two additional reconstructed female genotypes were each found in two separate progeny arrays. Thus, two females that we did not collect

also mated with at least two males each. Similarly, three females collected from Florida in August possessed genotypes that matched reconstructed maternal genotypes. Hence both Virginia and Florida populations are polygynandrous, an observation consistent with the data for dusky pipefish reported by Jones & Avise (1997b). Although the July and August collections were taken in the same seagrass meadow, no reconstructed female genotypes matched field-caught females across the two separate collections.

Females from the Virginia and Florida populations differed in the number of eggs transferred per copulation. Females from the Florida population produced significantly more embryos per successful mating event than did those from the Virginia population (FL =  $135.1 \pm 10.1$ ; VA =  $99.9 \pm 7.6$ ; Mann-Whitney U-test:  $\chi^2_{1,120} 4.3$ ,  $P = 0.038$ ). Hence, females exhibit a lower rate of multiple mating in Florida but transfer a larger number of eggs per mating event than do females in Virginia. The fact that every offspring has exactly one mother and one father, coupled with our estimate of male mating success, allows calculation of the mean mating success of females for a given sex ratio. The mean mating success for females (including females with zero mating success) is the product of the ratio of males to females and the mean mating success of all males. By this reasoning, the mean mating success, including non-mating females with zero mating success, for females in Virginia must be about 4.2 on average [(2.52 mates per male \* 150 males)/91 females], whereas the mean mating success for females in Florida is only about 0.75 mating events per female [(1.33 mates per male \* 36 males)/64 females], a dramatic difference between populations.

*Population size and density*

Mean Population size for the Virginia site was estimated to have 635 adult females using the modified Peterson-Lincoln mark-recapture method (Jones & Avise 1997b, Table 1). This result contrasts sharply with the estimates of 122 and 162 adult females in the July and August Florida collections, respectively (Table 1). Given the observed adult sex ratios, these values translate into local breeding population sizes of 1682 adults for the Virginia population and 190 and 254 for the July and August Florida collections, respectively.

Direct measurements of population density based on the numbers of individuals collected given the area seined showed that the Virginia population was between three to six times as dense as the Florida populations (Table 1). This difference in density is similar to the difference in population size estimates based on the modified mark-recapture method, which showed a 6- to 9-fold higher number of breeding adults in Virginia. Overall, these results show clearly that dusky pipefish in the Virginia population occur at higher densities and exhibit larger local breeding populations than those in our Florida population (Table 1).

**Discussion**

Our results show that two genetically distinct populations of the dusky pipefish exhibit significant inter-population variation in the genetic mating system. Males from the Virginia population mate more frequently than males from the Florida population. The two populations also show significant differences in male reproductive success

despite similar number of embryos per mated male. This difference is driven primarily by the high frequency of unmated males in the Florida population. The observed variation in mating and reproductive success between populations translates directly into significant differences in intensity of sexual selection, as measurements of the genetic mating system using Bateman's principles indicate a greater potential for sexual selection on males in the Florida population. Other sources of evidence also support the notion that sexual selection on males is stronger in the Florida population. A less female-biased ASR is consistent with stronger sexual selection on males and weaker sexual selection on females in the Florida population.

Observed differences in the genetic mating systems of the two populations are likely driven by selection on male and female fecundity. Both populations show a significant trend for larger males to carry more eggs and have more mates per pregnancy than smaller males, suggesting that selection is acting on male body size. However, larger males may require more mating events to fill their brood pouches to capacity than smaller males. Since females from the Virginia population provide fewer eggs per mating event than females from the Florida population, more mating events are required to fill the brood pouch of Virginia males than those of Florida males. Thus, the difference in mating success may represent a fundamental difference in life history strategies between populations. One hypothesis that may explain these results is that the potential reproductive rate (number of offspring produced per unit of time) is different between the two populations (Ahnesjö 1995). For example, if the rate of egg production is lower in the Virginia population, it may be sufficient to explain the difference in

female reproductive contribution and hence male mating and reproductive success. Definitive resolution of this hypothesis awaits further detailed study on the mating behavior of females from the different populations.

*Temporal variation in the genetic mating system*

It is clear from other studies of syngnathid fishes that both the adult sex ratio and operational sex ratio vary throughout the breeding season, providing the potential for the intensity of sexual selection to vary temporally (Vincent et al. 1995). In earlier studies of Atlantic and Gulf Coast populations conducted throughout the year, females on average tended to be more abundant than males (Brown 1972, Mercer 1973). However, the adult sex ratios tend to show a higher proportion of males during the summer (June to August) than during the fall and winter months (September to April), presumably because males depart the seagrass beds for deeper waters earlier than females (Brown 1972, Mercer 1973). An alternative hypothesis is that males may suffer higher mortality than females, as has been observed in other species with high paternal investment in offspring (Forsgren et al. 2004).

While previous studies (Brown 1972; Mercer 1973; Vincent et al. 1995) of syngnathids have shown that adult and operational sex ratios can vary throughout the year, we did not find temporal variation in several population parameters between the two sampling periods of the Florida collections. The collections made in the same seagrass bed during July and August of 2003 showed similar ASRs and OSRs, as well as similar population densities and population sizes (Table 1). Genetic mating system

parameters such as  $\bar{X}_{ms}$ ,  $\bar{X}_{rs}$ ,  $I$ ,  $I_s$ , and  $\beta_{ss}$  also did not differ significantly between the two time periods. These data suggest that population demographics and the genetic mating system are locally stable over short periods of time.

Between-year temporal variation also has the potential to contribute to meaningful variation in the genetic mating system. However, the genetic mating system also shows a pattern of long-term stability in *S. floridae*. An earlier study of the St. Joseph Bay site by Jones & Avise (1997b) in July of 1994 revealed a similar OSR and estimated population size of females despite dissimilar ASRs (Table 1). The Bateman gradient,  $\beta_{ss}$ , and the number of mates per male also show surprisingly similar estimates between years (Table 3) despite differences in body size (SL), mean number of eggs transferred per copulation by females and mean number of eggs per male. In the Florida 1994 collection, adults were significantly larger, females transferred significantly more eggs per copulation and males had more viable embryos than in the 2003 collections (Jones & Avise 1997b). The differences in number of eggs transferred per copulation and male reproductive success are a direct result of the larger body size of the 1994 collections and may represent variable growth rates among years. In addition, the differences in body size may also be related to the differences in  $I$  and  $I_s$  between the 1994 and 2003 samples. Thus, even though we see some interesting temporal changes in life history and mating parameters across a nine-year period in the Florida population, the among-population differences appear to be much larger than the temporal differences. Nevertheless, this study suggests that the study of temporal variation in pipefish mating systems could be a fruitful area for future research.

*The effect of population density on the genetic mating system*

Higher population densities may facilitate greater rates of multiple mating by both sexes as a consequence of a higher rate of mate encounter (Kokko & Johnstone 2002, Kokko & Rankin 2006). In the present study, the Virginia site exhibited a three to six fold higher population density than the Florida population. Our results are consistent with the prediction that higher population densities result in higher rates of multiple mating since the Virginia population had higher mating and reproductive success. We also observed evidence for significantly stronger sexual selection on males in the less dense Florida population. Lower population density may make it difficult for small, unattractive males to find suitable mates, because larger females probably prefer to mate with larger males, as has been observed in *S. typhle* (Berglund et al. 1988).

While our study represents a comparison of only two populations and hence cannot resolve whether population density affects the genetic mating system of *S. floridae* by itself, it is instructive to consider our results in light of other comparative studies of mating systems as a function of population density. For example, Soucy & Travis (2003) found that rates of multiple paternity were higher in populations with greater densities of adult individuals in the least killifish, *Heterandria formosa*. Several studies of other taxa also have shown a positive relationship between rates of multiple mating and population density. For example, in some species of birds, increasing breeding density appears to be associated with a higher rate of extra-pair paternity at the within-species level (Møller 1991; Reyer et al. 1997; Westneat & Sherman 1997; Richardson & Burke 2001; Charmantier & Perret 2004; Mougeot 2004). However, other



studies in a variety of taxa have failed to find a positive relationship between population density and multiple paternity. In several species of birds, for example, there appears to be no relationship between extra-pair fertilization frequency and nesting density (Dunn et al. 1994; Conrad et al. 2001; Veiga & Boto 2000; Ratti et al. 2001). Similarly, population density was not positively related to the frequency of concurrent multiple paternity in *Drosophila melanogaster* (Ochando et al. 1996). Very few studies thus far have focused on non-avian taxa, so additional research on the relationship between population density and mating patterns in a wider variety of taxa is clearly warranted.

*Other environmental factors that may affect the genetic mating system*

In addition to population density, a wide range of other parameters could conceivably contribute to the observed variation in mating patterns between our populations. Differences in mating preferences among males and females with respect to body size (Berglund et al. 1988), parasite load (Rosenqvist & Johansson 1995), or ornaments (Berglund & Rosenqvist 2001) may contribute to variation in local genetic mating systems. A large number of biotic and abiotic environmental factors, such as food availability (Kvarnemo 1997) and predation (Kelly et al. 1999, Bronikowski et al. 2002), may also shape genetic mating systems. Probably the most important abiotic environmental parameter for pipefish reproduction is water temperature. Previous investigations of *S. typhle* show that as little as a 4°C difference in temperature regimes has a large effect on the potential reproductive rates of males and females (Ahnesjö 1995, Kvarnemo & Ahnesjö 1996). Our Virginia population occupied a site with lower

mean water temperatures than those experienced by the Florida population. The yearly temperature for the Chesapeake Bay near the York River, Virginia averages 15°C (data available from the Chesapeake Bay Observing System, [www.cbos.org](http://www.cbos.org)), whereas the yearly surface water temperature of Panama City Florida (~50 km from St. Joe Bay) averages 20°C (data available from Environmental Protection Agency, <http://www.epa.gov/storet/index.html>). Additional studies, possibly including common garden mating experiments, will be needed to resolve the effects of temperature on mating patterns in this species.

### *Conclusions*

Understanding the biotic and abiotic factors affecting genetic mating systems is a central goal of much research in evolutionary biology and behavioral ecology. Our study contributes to this goal by providing empirical evidence that critical mating system parameters, such as  $\bar{X}_{ms}$ ,  $\bar{X}_{rs}$ ,  $I$ ,  $I_s$ , and  $\beta_{ss}$ , can vary among populations within a species. Such variation may underlie divergent evolutionary trajectories for population-specific morphology, and thus play a significant role in the process of speciation. Much more work on geographic variation in genetic mating systems of this and other species will be required for a complete understanding of the ecological factors contributing to mating system evolution.

**CHAPTER IV**

**GEOGRAPHIC VARIATION IN THE GENETIC MATING SYSTEM OF THE  
DUSKY PIPEFISH, *SYNGNATHUS FLORIDAE*. III: INFLUENCE OF  
DEMOGRAPHIC AND ENVIRONMENTAL FACTORS**

**Introduction**

Current quantitative theory of animal genetic mating systems can be characterized as the populational response to selection in terms of variation in mating and reproductive success (Arnold 1994, Shuster & Wade 2003). This approach emphasizes processes that influence selection on the morphological and behavioral traits that are directly responsible for the acquisition of mates, fecundity and fitness of individuals (Shuster & Wade 2003). The appeal of such an approach is that it applies a statistically rigorous framework for testing hypotheses concerning the evolution of traits as a consequence of sexual selection (Shuster & Wade 2003). Thus far, studies using this quantitative approach have validated such techniques (Jones et al. 1999, Jones et al. 2002, Jones et al. 2004, Jones et al. 2005, Mills et al. 2007) and have shown their flexibility to the application of a host of evolutionary processes (Shuster & Wade 2003).

Despite this recent progress in theory, only a handful of studies have investigated the effects of specific ecological factors on mating and reproductive success. The length and synchrony of the breeding season (Shuster & Wade 2003, Spottiswoode and Møller 2004), local population density (Lloyd 1967, Griffith et al. 2002, Prohl 2002), the operational sex ratio (Kvarnemo & Ahnesjö 1996, Prohl 2002, Jones et al. 2005, Mills et al. 2007), and predation (Kelly et al. 1999, Bronikowski et al. 2002, Lodé et al. 2004)

have all been shown to influence mating success at the population level. Ecological factors may also set limits on reproductive success. For instance, temperature (Ahnesjö 1995, Kvarnemo 1997, Fischer et al. 2003), resource abundance (Kvarnemo 1997, Turner & McCarty 1998), habitat structure and fragmentation (Turner & McCarty 1998, Aguilar & Galetto 2004), parasite load (Fitze et al. 2004), resource competition (Martin & Martin 2001) and predation (Bronikowski et al. 2002) all may influence variation in offspring production. These studies represent the beginning of a more complete understanding of mating system organization that encompasses ecological conditions that are both environmental and demographic in nature.

One method to partition the relative affects of ecological factors on genetic mating system components is to sample multiple geographically isolated populations that experience disparate ecological regimes. In this manner, population-level responses to ecological conditions can be measured under natural conditions. However, only a handful of studies that have investigated variation in mating systems on broad geographical scales exist and they have not provided clear trends with respect to mating system organization among sites. For example, a few studies that investigated the degree of multiple mating have found little or no variation between geographically distant populations (Jones et al. 2001b, Goodisman et al. 2002). In contrast, other studies have provided evidence that there are significant differences between mating and reproductive success among distant populations, although the ecological factors responsible for such differences are not yet well understood (Weatherhead & Boag 1997, Griffith et al. 1999). Finally, a handful of studies appear to have documented variation

in mating patterns and to have pinpointed the possible causal factors leading to the differences among populations. For example, differences in mating success appear to be correlated with female size in sailfin mollies (Trexler et al. 1997). Finally, one study has clearly shown a positive relationship between an ecological parameter (predation) and a mating system parameter, the frequency of multiple insemination, in Trinidadian guppies (Kelly et al. 1999, Bronikowski et al. 2002).

The goal of this study is to elucidate the combined effects of specific ecological phenomena that influence genetic mating system parameters in the dusky pipefish, *Syngnathus floridae*. The dusky pipefish is widely distributed in the western Atlantic Ocean and Gulf of Mexico (Dawson 1982). Populations of *S. floridae* are now known to have different morphological characteristics and have been shown to experience different demographic regimes with respect to population density, adult sex ratios, operational sex ratios and adult population sizes. In addition, different populations are characterized by quantitative differences in their genetic mating systems (**CHAPTER III**). Populations of *S. floridae* also likely encounter a wide range of environmental conditions such as temperature, salinity, turbidity and seagrass habitat that may also have direct effects on individual fitness, mating and reproductive success.

To study the effects of specific ecological conditions on genetic mating system correlates, adult collections of dusky pipefish were made in five locations from both the Atlantic coast of North America and the Gulf of Mexico. These five sites differed in several abiotic environmental conditions such as mean yearly water temperature and turbidity, and experienced disparate demographic regimes in adult sex ratios, adult

population density and adult population size. Multiple regression analysis was used to partition the effects of specific environmental, morphological and demographic factors on male and female genetic mating system correlates including Bateman's three principles, the opportunity for selection ( $I$ ), the opportunity for sexual selection ( $I_s$ ), and the Bateman gradient ( $\beta_{ss}$ ).

## Materials and Methods

### *Sample collection*

Adult dusky pipefish were collected during the summer mating season (June – August) from five sites between July 2003 and August 2005. Two of the sites were from the Atlantic coast (VA and NC) and three were from the Gulf of Mexico (TB, SJ, TX; Fig. 2.1, Table 2.1). These five sites have previously been shown to show strong isolation by distance with respect to population genetic structure (**CHAPTER II**). The TB and SJ sites were revisited approximately one month after the first sample was taken to improve the sample size. Individuals were captured at each location by seine net (2 mm mesh) during low tide from measured plots marked with stakes inside shallow (< 1m) seagrass meadows. Each plot was completely seined a minimum of three full sweeps or until a full sweep of the area captured less than five percent of the original sweep. Individuals were assessed for sex and maturity by criteria outlined in **CHAPTER III**. All adults captured were measured for standard length (SL) to the nearest mm and all females were measured for body depth (BD) with calipers to the nearest mm. Males were assessed for pregnancy and stage of development of embryos.

Pregnant males that did not have sufficient brood development for genetic analyses were maintained in buckets of seawater supplied with an air stone and fed freshly hatched *Artemia* nauplii. Once embryos developed eyespots, pregnant males were sacrificed for parentage analysis. All other adults were sacrificed in the field by severing their spinal column above the operculum and preserved in 95% ethyl alcohol.

#### *Abiotic environmental data collection*

Environmental data were collected at each site visitation. Measured environmental variables included temperature, salinity, and turbidity. Mean, maximum, and minimum yearly sea surface water temperatures were calculated from mean monthly temperatures using data available from U.S. government agencies (VA: The Chesapeake Buoy Observing System, [www.cbos.org](http://www.cbos.org); TB, SJ, NC: The Environmental Protection Agency, [www.epa.gov/storet/index.html](http://www.epa.gov/storet/index.html); TX: the Department of Nearshore Research/Texas Coastal Ocean Observation Network <http://lighthouse.tamucc.edu/pq>). Field measurements of temperature were measured with a standard mercury thermometer, salinity was measured with a refractometer, and turbidity was measured with a Secchi disk.

#### *Seagrass data collection*

Mean seagrass percent cover, shoot height, shoot density, above ground biomass, and percent composition were measured for each seagrass species encountered at the five sampling sites on the first visitation. At each site a 0.5 X 0.5 m quadrat (0.25 m<sup>2</sup>)

divided into 25 0.1 X 0.1 m (0.1m<sup>2</sup>) sections was randomly thrown into the area staked for fish collection. Five sections were selected using a random number table and all vegetation inside each selected section was harvested above the sediment level. This process was repeated for a total of five replicates at each site. All plant material above the rhizomes was then placed in individual Ziploc<sup>®</sup> bags, placed on ice and transferred to a -20°C freezer until processing. For processing, seagrass shoots were thawed, rinsed in fresh water, and separated by species. To calculate mean shoot height, at least ten shoots of each seagrass species were randomly selected and measured lengthwise to the nearest mm. If less than 10 shoots were encountered, all shoots were measured. The remainder of the shoots were then counted and averaged for each quadrat to calculate mean shoot density (shoots per m<sup>2</sup>) for each species and total species. All epiphytes were cleaned off each shoot and all seagrass material for each species was dried in a 55°C incubator for 24 hours before determining dry mass for biomass estimates for each quadrat.

#### *Demographic data collection*

In order to assess the influence of demographic features on the genetic mating system of dusky pipefish, several population-level measurements were investigated, including the adult sex ratio (ASR), the operational sex ratio (OSR), population density, and population size. The adult sex ratio (ASR) is given as the number of adult males divided by the total number of adults collected, whereas the operational sex ratio (OSR) is the ratio of non-pregnant males to non-pregnant males plus adult females (Kvarnemo & Ahnesjö 1996). Population density amounted to the total number of individuals



caught per m<sup>2</sup> for each sex and for the total number of adults. A modified Lincoln-Peterson method of capture-recapture was used to estimate female local population size in all samples based on number of matched reconstructed female genotypes to field caught individuals (Jones & Avise 1997b, **Methods CHAPTER III**).

#### *Parentage analysis and genetic mating system correlates*

Individual adult male and female *S. floridae* from each population were genotyped using four polymorphic microsatellite DNA loci previously employed to characterize the mating system in *S. floridae* (Jones & Avise 1997b). Brood pouches were dissected and genotyped according to protocols in the **Methods** section of **CHAPTER III**. Parentage analysis was conducted according to protocols in the **Methods** section of **CHAPTER III**. Several measures of the genetic mating system, including the mean reproductive success ( $\bar{X}_{ms}$ ), mean reproductive success ( $\bar{X}_{rs}$ ), and the three Bateman's principles, i.e., the opportunity for selection ( $I$ ), the opportunity for sexual selection ( $I_s$ ) and the Bateman gradient ( $\beta_{ss}$ ), were calculated following protocols outlined in the **Methods** section of **CHAPTER III**.

#### *Statistical analysis*

Statistical analyses were performed with JMP IN™ v5.1 statistical software package (SAS Institute Inc., Cary NC). All statistics were analyzed first for normality and equal variances. If these assumptions were not met, data were transformed or if no transformation satisfied a priori assumptions, appropriate non-parametric tests were

applied. Statistical tests as well as any transformations are indicated throughout the text. All means are reported  $\pm$  one standard error of the mean (SE).

A stepwise multiple linear regression model (Sokal & Rohlf 1995) was used to test for significant relationships between mating system parameters and morphological, environmental and demographic criteria pooled for the five sites. Environmental data were averaged for the two TB and two SJ collections and samples were combined for genetic mating system correlates. Estimates of total population density were calculated by multiplying the female population size by the adult sex ratio to first get an estimate of male population size. The male and female population sizes were then summed to estimate total population density for each site. The criterion for adding steps to the regression model was  $P = 0.25$ , and retaining steps  $P = 0.10$ . Because the criterion for retaining steps was greater than  $P = 0.05$ , it allowed for best-fit regression line construction that included some non-significant individual regressions (Sokal & Rohlf 1995). Prior to final fitting of models, these non-significant regressions were removed and only significant partial regressions are reported.

## Results

### *Morphological differences between sites*

The five sites sampled in this study showed significant differences in adult mean body size (SL) pooled between all samples for each site and after natural log transformation of data to fit normality and equal variance assumptions (ANOVA:  $F_{4,581} = 72.83$ ,  $P < 0.0001$ , Fig. 4.1). The largest body sizes for adult male and female *S.*

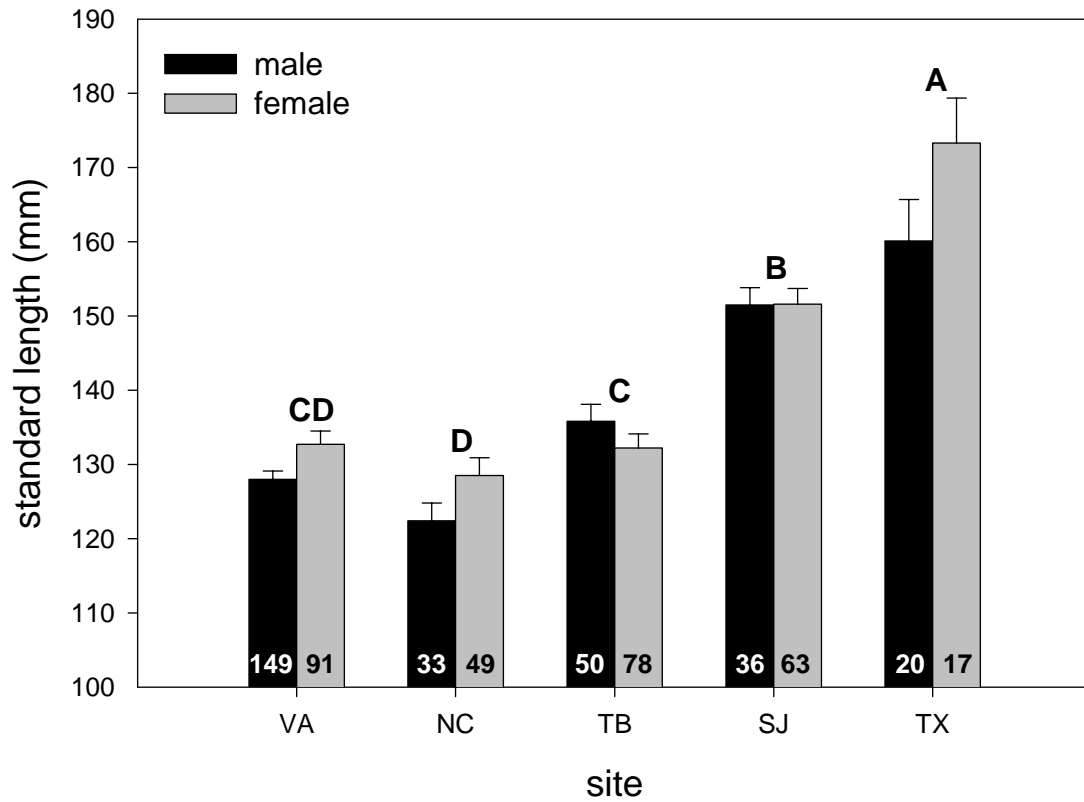


Figure 4.1. Mean standard length ( $\pm$  SE) of male and female dusky pipefish captured from different sampling sites (Table 2.1, Fig. 2.1). Numbers at the base of each bar represent the sample size. Bars with like letters have adult population means that were not significantly different from one another (Tukey-Kramer HSD test,  $\alpha = 0.05$ ).

*floridae* were encountered in the TX site ( $166 \pm 4$  mm). Intermediate body sizes were encountered in the TB and SJ collection and there was no significant difference in SL between sexes in either the TB or SJ samples (2 way ANOVA: TB sex  $F_{1,125} = 3.34$ ,  $P = 0.07$ ; SJ sex  $F_{1,96} = 0.007$ ,  $P = 0.97$ ). However, there were significant differences between the collection times (2-way ANOVA: TB time  $F_{1,125} = 18.97$ ,  $P < 0.0001$ ; SJ time  $F_{1,96} = 7.53$ ,  $P = 0.007$ ). These differences in SL amounted to an 8mm increase in the adult SL between the July and August sample in SJ (July  $148 \pm 2$  mm; August  $156 \pm 2$  mm) and a 9mm increase in adult SL between the June and July sample in TB (June SL  $131 \pm 1$  mm; July SL  $142 \pm 2$  mm). The smallest adult SL was recorded in the VA and NC samples (VA  $129 \pm 1$  mm; NC  $126 \pm 1.6$ ). The only significant sexual dimorphism in body length was recorded in the VA sample where males were, on average, 5mm smaller than females (ANOVA<sub>1,238</sub> = 5.94,  $P = 0.016$ ).

#### *Abiotic environmental data*

The five sites investigated in this study were different with respect to the abiotic environmental criteria measured. Mean, maximum, and minimum yearly water temperatures were most similar between the NC and SJ sites as well as between the TB and TX sites (Table 4.1). The VA site had the lowest estimates for mean, maximum and minimum yearly water temperature. All sites yielded temperatures near the maximum yearly water temperature during the time of collection (Table 4.1). Salinity was variable between sites and between sampling times within sites. The highest salinities were

Table 4.1. Summary of abiotic environmental data. Calculated yearly water temperature (mean, maximum and minimum) and water temperature, salinity and turbidity (Secchi disk depth) measured at each of the five sites.

Site	WATER TEMP <sup>1</sup>			Date	FIELD DATA		
	Mean (°C)	Max (°C)	Min (°C)		Water temp (°C)	Salinity (‰)	Secchi disk depth (m)
VA	15	31	0	8/03	29.5	15	0.4
NC	19	34	3	7/04	29.0	28	0.7
TB	24	31	13	6/04	30.5	28	1.5
				7/04	31.0	32	1.2
SJ	20	32	6	7/03	29.0	15	2.1
				8/03	30.5	25	1.8
TX	24	33	11	8/05	33.0	36	0.6

<sup>1</sup>Maximum, minimum and mean yearly surface water temperatures are based on monthly averages recorded by the following: Mid Bay Buoy recorded from 7/01/02 – 7/01/03 for the VA site (data available from the Chesapeake Bay Observing System, [www.cbos.org](http://www.cbos.org)); Neuse River estuary approximately 40km NNE of Morehead City recorded from 1/01/05 – 12/31/05 for the NC site (data available from EPA, <http://www.epa.gov/storet/index.html>); Dunedin Sound recorded 12/31/03 – 11/01/04 for the TB site (data available from EPA, <http://www.epa.gov/storet/index.html>); St. Andrews Bay situated approximately 50km NW from St. Joseph Bay recorded 1/01/03 – 12/31/03 for the SJ site (data available from EPA, <http://www.epa.gov/storet/index.html>); Port Aransas recorded from 1/01/05-12/31/05 for the TX site (data available from the Department of Nearshore Research/Texas Coastal Ocean Observation Network <http://lighthouse.tamucc.edu/pq>).

recorded in the TX site and the lowest in the VA and July SJ site (Table 4.1). Turbidity is negatively correlated with light penetration and Secchi disk depth. The highest Secchi disk depth and hence the least turbidity was recorded at the SJ site. The smallest Secchi disk depth was recorded in the VA site and intermediate Secchi disk depths were found in the NC, TX and TB sites (Table 4.1).

### *Seagrass estimates*

Collection sites differed in composition, percent cover, mean shoot height, mean shoot density and mean biomass of living seagrass (Table 4.2). Seagrasses encountered at the Atlantic sites included eelgrass, *Zostera marina*, and shoal grass, *Halodule wrightii*. Seagrasses encountered in the Gulf of Mexico populations included *H. wrightii*, manatee grass, *Syringodium filiforme*, and turtle grass, *Thalassia testudinum*. A sand/silt substrate and *Z. marina* characterized the VA site. A sand/silt substrate and a mixed community comprised of *H. wrightii* and *Z. marina* characterized the NC site. The TB site had a sand/silt substrate and was primarily dominated by *S. filiforme*. The SJ site had a substrate comprised of shell hash and sand and was dominated by *T. testudinum*. The TX site was also dominated by *T. testudinum* and had a substrate of shell hash and silt. All sites had nearly 100% cover of living seagrass, except the NC site, which had small patches of seagrass interspersed with bare substrate (Table 4.2). Mean shoot height was greatest in the TX and TB sites, which yielded similar estimates. Similar intermediate mean shoot height estimates were encountered the SJ and NC samples. The lowest mean shoot height was encountered in the VA site. Seagrass shoot

Table 4.2. Summary of seagrass data for collection sites. Listed are substrate, species composition, percent cover (% Cover), mean shoot height (mm  $\pm$  SE), shoot density (shoots m<sup>-2</sup>  $\pm$  SE) and biomass (g dry weight m<sup>-2</sup>  $\pm$  SE) calculated for each species of seagrass and totaled for all species at each site.

Site	Date	Substrate	Species	% Cover	Mean shoot height	Shoot density	Seagrass biomass
VA	8/03	sand/silt	<i>Z. marina</i>	92	90.1 $\pm$ 18.0	4248 $\pm$ 625	72.3 $\pm$ 26.3
NC	7/04	sand/silt	<i>H. wrightii</i>	56	117.6 $\pm$ 21.4	8460 $\pm$ 5115	65.0 $\pm$ 40.8
			<i>Z. marina</i>	56	131.6 $\pm$ 2.1	3304 $\pm$ 1478	58.5 $\pm$ 26.1
			Total	60	124.6 $\pm$ 11.7	11764 $\pm$ 5977	123.5 $\pm$ 56.7
TB	6/04	sand/silt	<i>S. filiforme</i>	100	152.6 $\pm$ 14.8	7188 $\pm$ 1837	114.8 $\pm$ 41.5
			<i>H. wrightii</i>	52	185.4 $\pm$ 45.9	1096 $\pm$ 624	5.4 $\pm$ 2.5
			Total	100	169.0 $\pm$ 29.6	8284 $\pm$ 1617	120.2 $\pm$ 40.0
SJ	7/03	sand/ shell hash	<i>T. testudinum</i>	100	144.8 $\pm$ 10.7	2548 $\pm$ 219	146.1 $\pm$ 10.6
			<i>S. filiforme</i>	44	72.1 $\pm$ 7.4	844 $\pm$ 514	6.1 $\pm$ 3.7
			Total	100	120.4 $\pm$ 8.1	3392 $\pm$ 423	152.3 $\pm$ 12.8
TX	8/05	silt/shell hash	<i>T. testudinum</i>	96	201.8 $\pm$ 25.2	3888 $\pm$ 880	135.0 $\pm$ 34.5
			<i>H. wrightii</i>	60	118.0 $\pm$ 3.8	3288 $\pm$ 1814	31.0 $\pm$ 19.8
			Total	96	170.0 $\pm$ 16.6	7176 $\pm$ 1031	166.0 $\pm$ 20.1

density (shoots per m<sup>2</sup>) was highest in the NC site and lowest at the VA and SJ sites.

Seagrass biomass (g dry weight per m<sup>2</sup>) was highest in the TX and SJ sites and lowest in the VA site.

### *Demographic data*

All sites had a slightly male-biased ASR except for the VA and TX sites where more males were caught than females (Table 4.3). Only the VA site had an ASR that had a significant departure from equality ( $\chi^2$ :  $P < 0.01$ ). In sites that were sampled more than once (TB, SJ), the ASR was similar for all samples. The OSR, on the other hand, was highly variable among temporal samples (Table 4.3). All sites except for the TX sample had a significantly male-biased OSR (Table 4.3). Population density (individuals per m<sup>2</sup>) measured in the field was highest in the NC sample and lowest in the SJ and TX sites (Table 4.3). Population density was not estimated for the July TB sample. Adult female population size was estimated for each sampling time using the Lincoln-Peterson mark-capture-recapture technique (Jones & Avise 1997b) except the July TB sample since no reconstructed female genotypes matched individuals captured in the field. The VA site yielded the highest estimate of adult female population size whereas the TX site had a very small adult female population size (Table 4.3).

### *Male mating behavior*

An evaluation of the July and August SJ collections revealed no significant differences in the number of mates, number of embryos and SL per mated male between



Table 4.3. Summary of demographic data for each collection site. Listed are sample sizes of adults ( $n$ ), males ( $m$ ), non-pregnant males ( $m'$ ) and females ( $f$ ), adult sex ratios (ASR; ratio of males to total adults), operational sex ratios (OSR; ratio of non-pregnant males to females + non-pregnant males), population density (ind m<sup>-2</sup>) and mean female population size (estimated using the modified Lincoln-Peterson method with 95% CE) of adult *S. floridae* from different sample collections.

Site	Date	Sample Size				ASR $m / n$	OSR $m'/(f+m')$	Population density (ind m <sup>-2</sup> )			Adult Female Population Size (95% CE)
		$n$	$m$	$m'$	$f$			Total	$m$	$f$	
VA	8/03	241	150	3	91	0.62**	0.03***	0.18	0.11	0.07	635 (323-947)
NC	7/04	82	33	7	49	0.40	0.13***	0.27	0.07	0.20	87 (68-105)
TB	6/04	92	37	8	55	0.40	0.13***	0.11	0.04	0.06	146 (76-216)
	7/04	36	13	0	23	0.36	0.00***	---	---	---	---
SJ	7/03	53	19	6	34	0.36	0.15***	0.06	0.02	0.04	122 (52-191)
	8/03	47	17	6	30	0.36	0.17**	0.03	0.01	0.02	162 (42-281)
TX	8/05	37	20	5	17	0.54	0.22	0.06	0.03	0.03	47 (24-70)

\*\* Denotes significant departure from equality ( $\chi^2$ :  $p < 0.01$ )

\*\*\* Denotes significant departure from equality ( $\chi^2$ :  $p < 0.001$ )

sampling times and these collections were therefore combined for analysis (**CHAPTER III**, Table 4.4). Similarly the June and July collection at the TB site did not significantly differ in the number of embryos per mated male (ANOVA:  $F_{1,28} = 1.05$ ,  $P = 0.31$ ). However these two collections significantly differed in number of mates (Mann-Whitney U-test:  $\chi^2 = 14.20$ , 1 d.f.,  $P > 0.0002$ ) and SL of mated males (ANOVA:  $F_{1,28} = 10.13$ ,  $P < 0.004$ ). This variation may be due to the small sample size ( $n = 9$ ) of mated males during this sampling time. Because of the small sample size encountered in the second collection and because reconstructed female genotypes from the first collection matched females caught in the second collection, these collections were assumed to be the same breeding population and therefore the two TB estimates were pooled between samples for mating system estimates.

Males differed among sites in the maximum number of mates and the number of mates per mated male, but not in the number of embryos per mated male among sites (Table 4.4). The highest numbers of mates per mated male were found in the VA site, and this estimate was significantly higher than all other sites (ANOVA:  $F_{4,118} = 21.97$ ,  $P < 0.0001$ , Tukey-Kramer post hoc analysis:  $\alpha = 0.05$ , Table 4.4). The TX, TB and NC sites had the fewest number of mates per mated male and were not significantly different from one another in this estimate. The SJ site had intermediate numbers of mates per mated male and was significantly different from all other sites except the NC site. Estimates of the number of embryos per mated male were not significantly different between sites (Tukey-Kramer post hoc analysis:  $\alpha = 0.05$ ). The highest number of

Table 4.4. Summary statistics for pregnant males analyzed for parentage. Site, number of males analyzed ( $n_m$ ), max number of mates (max), mean number of mates (mates), mean number of embryos (embryos) and mean standard length (SL) are listed.

<b>Site</b>	<b>Date</b>	<b><math>n_m</math></b>	<b>Max</b>	<b>Mates (<math>\pm</math> SE)</b>	<b>Embryos (<math>\pm</math> SE)</b>	<b>SL (mm <math>\pm</math> SE)</b>
<b>VA</b>	8/03	30	4	$2.60 \pm 0.13$	$259.7 \pm 16.4$	$132.0 \pm 2.2$
<b>NC</b>	7/04	22	3	$1.75 \pm 0.14$	$197.0 \pm 18.4$	$124.2 \pm 2.4$
<b>TB</b>	6/04	21	2	$1.24 \pm 0.10$	$194.2 \pm 20.1$	$134.0 \pm 2.1$
	7/04	9	2	$2.00 \pm 0.00$	$228.3 \pm 19.2$	$145.7 \pm 2.6$
<b>SJ</b>	7/03	11	3	$2.18 \pm 0.2$	$265.9 \pm 33.7$	$150.4 \pm 3.9$
	8/03	11	4	$1.82 \pm 0.3$	$274.5 \pm 30.8$	$153.9 \pm 3.0$
<b>TX</b>	8/05	13	2	$1.31 \pm 0.16$	$256.2 \pm 38.3$	$158.2 \pm 4.8$

embryos per mated male was encountered in the SJ site and the lowest was encountered in the NC site (Table 4.4).

Significant differences were detected for SL of mated males between sample sites (Kruskal Wallance Rank Sums Test:  $\chi^2 = 51.25$ , 4 d.f.,  $P < 0.0001$ , Table 4.4). Pregnant males were largest in the TX and SJ site and smallest in the NC site (Table 4.4). The relationship between the number of embryos per unit of SL in males was not significantly different between sites (site\*SL ANCOVA:  $F_{4,109} = 0.13$ ,  $P = 0.97$ ) and this relationship was significantly positive (SL ANCOVA:  $F_{1,113} F = 73.55$ ,  $P < 0.0001$ ). Males had significantly more embryos on average in the SJ and NC sites, whereas the TX sample had the fewest number of embryos (site ANCOVA:  $F_{4,113} = 6.52$ ,  $P < 0.0001$ ). The relationship between number of mates and SL was not significantly different among sites (site\*mates 2-way ANOVA:  $F_{4,104} = 1.03$ ,  $P = 0.39$ ) and the relationship was significantly positive (mates 2-way ANOVA:  $F_{1,113} = 35.46$ ,  $P < 0.0001$ ). According to this two-factor ANOVA, the highest numbers of mates per pregnant male were found in the NC and VA sites and the lowest number of mates per pregnant male was found in the TX site.

Estimates of male mean mating ( $\bar{X}_{ms}$ ) and reproductive success ( $\bar{X}_{rs}$ ) differed between sites. Males from the VA site had a significantly higher  $\bar{X}_{ms}$  than all other sites that shared similar values of  $\bar{X}_{ms}$  (ANOVA:  $F_{4,140} = 11.46$ ,  $P < 0.0001$ ; Table 4.5). Males also had significantly higher mean reproductive success,  $\bar{X}_{rs}$ , in the VA site than in the NC site (ANOVA:  $F_{4,140} = 2.62$ ,  $P < 0.04$ ). This difference in  $\bar{X}_{rs}$  was not

Table 4.5. Quantitative characterization of male mating system estimates. Shown estimates are mean mating success ( $\bar{X}_{ms}$ ), the variance in mating success ( $\sigma_{ms}$ ), the opportunity for sexual selection ( $I_s$ ), mean reproductive success ( $\bar{X}_{rs}$ ), the variance in reproductive success ( $\sigma_{rs}$ ), the opportunity for selection ( $I$ ), and the Bateman gradient ( $\beta_{ss} \pm SE$ ).

Site	Date	<i>n</i>	Mating Success			Reproductive Success			
			$\bar{X}_{ms}$	$\sigma_{ms}$	$I_s$	$\bar{X}_{rs}$	$\sigma_{rs}$	$I$	$\beta_{ss} (\pm SE)$
VA	8/03	31	$2.52 \pm 0.17$	0.7914	0.13	$251.3 \pm 21.5$	9165.8	0.15	$0.25 \pm 0.07$
NC	7/04	33	$1.40 \pm 0.17$	0.8690	0.44	$157.6 \pm 21.8$	13040.5	0.53	$0.52 \pm 0.11$
TB	6-7/04	35	$1.26 \pm 0.16$	0.4908	0.31	$175.2 \pm 18.6$	11237.7	0.37	$0.65 \pm 0.10$
SJ	7-8/04	31	$1.33 \pm 0.16$	1.3542	0.76	$180.2 \pm 20.8$	23911.7	0.74	$0.59 \pm 0.08$
TX	8/05	16	$1.06 \pm 0.19$	0.4625	0.41	$208.2 \pm 40.5$	25561.6	0.59	$0.95 \pm 0.16$

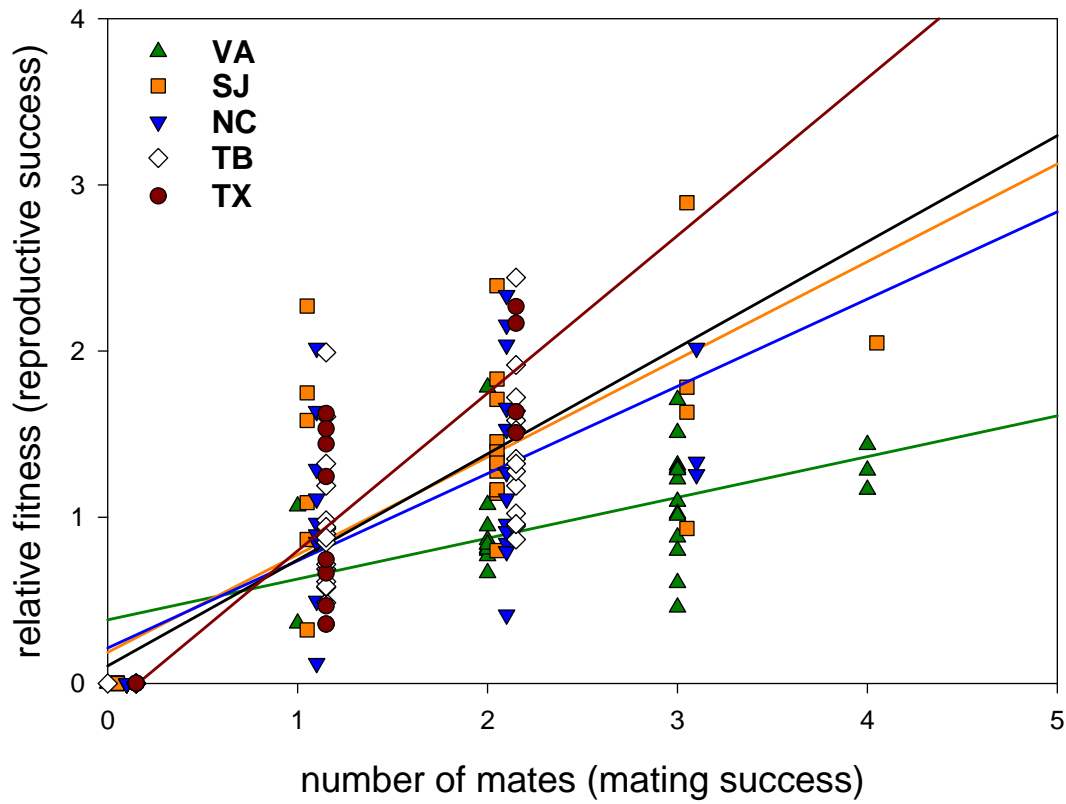


Figure 4.2. Relationship between reproductive success and mating success for males from different sampling sites. Reproductive success is shown as relative fitness. The sexual selection ( $\beta_{ss}$ ) gradient is shown as the colored weighted least-squares regression line for each site.

Table 4.6. Summary statistics for females. Listed are the number of unique females caught ( $f$ ), mean standard length (SL), mean body depth (BD), number of unique female reconstructed genotypes ( $f_c$ ), number of reconstructed female genotypes matched to individuals caught in the field ( $f_r$ ), percent recaptured ( $\%_r$ ), probability of identity for females caught ( $P_{ID-f}$ ), the probability of identity for reconstructed genotypes ( $P_{ID-fr}$ ), number of mates for individual females constructed from recaptured females, number of identical female reconstructed genotypes matching reconstructed genotypes (in parentheses), and the mean female reproductive contribution per mated male ( $FRC \pm SE$ ).

Site	Date	$f$	SL	BD	$f_c$	$f_r$	$\%_r$	$P_{ID-f}$	$P_{ID-fr}$	Mates				FRC
			(mm $\pm$ SE)	(mm $\pm$ SE)						1	2	3	4	
VA	8/03	91	132.7 $\pm$ 1.7	5.2 $\pm$ 0.1	75	10	11	8.1 X 10 <sup>-5</sup>	3.5 X 10 <sup>-5</sup>	10	(2)			99.9 $\pm$ 7.6
NC	7/04	49	128.5 $\pm$ 2.4	5.2 $\pm$ 0.1	34	19	39	8.5 X 10 <sup>-6</sup>	8.0 X 10 <sup>-6</sup>	12	6	1		112.6 $\pm$ 10.4
TB	6/04	55	128.9 $\pm$ 2.2	4.2 $\pm$ 0.1	20	7	13	2.2 X 10 <sup>-7</sup>	4.7 X 10 <sup>-7</sup>	3	3(5)	1		154.9 $\pm$ 15.7
	7/04	23	140.0 $\pm$ 3.4	5.2 $\pm$ 0.2	17	0	0							114.2 $\pm$ 13.3
SJ	7/03	34	148.1 $\pm$ 2.8	5.3 $\pm$ 0.2	20	5	15	8.6 X 10 <sup>-7</sup>	3.6 X 10 <sup>-7</sup>	3	2(2)			121.9 $\pm$ 15.7
	8/03	30	155.7 $\pm$ 3.1	6.2 $\pm$ 0.2	20	3	10			3				151.0 $\pm$ 17.2
TX	8/05	17	173.3 $\pm$ 4.0	6.9 $\pm$ 0.2	15	5	29	1.5 X 10 <sup>-5</sup>	2.6 X 10 <sup>-5</sup>	3	2			195.9 $\pm$ 37.8

encountered among any other sites (Tukey-Kramer post hoc analysis:  $\alpha = 0.05$ ). The genetic mating system was estimated using Bateman's three principles for each site (Table 4.5). Estimates of the opportunity for sexual selection ( $I_s$ ) and the opportunity for selection ( $I$ ) were significantly different among sites (Levene homogeneity of variance test  $I$ :  $F_{4,140} = 6.74$ ,  $P < 0.0001$ ;  $I_s$   $F_{4,140} = 5.03$ ,  $P < 0.0008$ ). An analysis of covariance revealed significant difference in the slope of the Bateman gradient ( $\beta_{ss}$ ) among sites (mates\*site ANCOVA:  $F_{4,135} = 4.23$ ,  $P < 0.003$ ). Estimates were highest in the TX site and lowest in the VA site (Table 4.5, Fig. 4.2). Both of these estimates were significantly different from the NC, TB and SJ that shared similar estimates of  $\beta_{ss}$ .

#### *Female mating behavior*

Females from the NC and VA sample had a high ratio of body depth (BD) to SL, whereas intermediate ratios were encountered in the SJ and TX site. The lowest ratio occurred in the TB site (Table 4.6). The slope of the relationship between BD and SL was similar in all sites except a significant interaction between BD and SL was discernable in the VA sample compared to all other sites (BD\*site ANCOVA:  $F_{4,288} = 4.35$ ,  $P = 0.002$ ). Modest recapture rates and low probabilities of identity (with respect to multi-locus microsatellite genotypes) for field-collected females and female genotypes reconstructed from progeny arrays allowed for the partial reconstruction of mating frequencies for females among sites (Table 4.6). The maximum number of mates reconstructed for any individual female was three. The evidence consisted of clutches of eggs attributable to the same female genotype that were present in the brood pouches of



three distinct males. Evidence for females mating with at least three males was found in both the TB and NC sites (Table 4.6). The female reproductive contribution (FRC) equal to the mean number of embryos contributed by females to pregnant males per mating event was significantly greater among TX and TB sites than in the VA site after natural log transformation (ANOVA:  $F =_{4,220} = 4.19$ ,  $P < 0.003$ ). The SJ and NC sites were not statistically different from TX, TB or VA sites with respect to female reproductive contribution (Tukey-Kramer post hoc analysis:  $\alpha = 0.05$ ).

#### *Multiple regression analysis*

Stepwise multiple linear regression analysis was used to predict mating system correlates as a function of specific morphological, environmental, and demographic phenomena (Tables 4.7 & 4.8). Because pairwise comparisons found significant correlations between mean yearly water temperature and minimum yearly water temperature, field temperature and maximum yearly temperature, adult population density and male and female densities, and adult population size and male and female population size, these variables were collapsed and only mean yearly water temperature, field water temperature, adult population density and adult population size were used in the final analysis. Other significant pairwise correlations such as the OSR and seagrass biomass were included in these analyses since these factors are not known to causally influence one another.

In all mating system predictors investigated, nearly all variance could be explained ( $R^2 > 0.85$ ) using the specific model criteria measured in this study except for

Table 4.7. Stepwise multiple linear regression models of ecological criteria loaded on female reproductive contribution (FRC), variance in male mating success ( $\bar{X}_{ms}$ ), and the variance in male reproductive success ( $\bar{X}_{rs}$ ). Slope (b) and  $R^2$  values of the partial regression ( $pR^2$ ) and model are listed. All values for the slope are significant at  $P < 0.05$  unless otherwise noted.

Criteria	FRC		$\bar{X}_{ms}$		$\bar{X}_{rs}$	
	b	$pR^2$	b	$pR^2$	b	$pR^2$
<b>Environmental</b>						
Temperature: field	16.76	0.78	-0.11	0.26		
Temperature: mean yearly						
Salinity						
Secchi disk depth						
Seagrass: mean height						
Seagrass: total shoot density						
Seagrass: total biomass	0.39	0.22				
<b>Morphological</b>						
Standard length: males						
Standard length: females						
Body Depth: females						
<b>Demographic</b>						
Sex ratio: adult			0.79	0.03	300.7	0.85
Sex ratio: operational						
Population density: adult						
Population size: adult			< 0.001	0.71		
<b>Model <math>R^2</math></b>		1.00		1.00		0.85

Table 4.8. Stepwise multiple linear regression models of ecological criteria loaded on the three Bateman's principles. The opportunity for sexual selection in males ( $I_s$ ), the opportunity for selection in males ( $I$ ) and the male Bateman Gradient ( $\beta_{ss}$ ) are dependant variables. Slope (b) and  $R^2$  values of the partial regression ( $pR^2$ ) and model are listed. All values for the slope are significant at  $P < 0.05$ .

Criteria	$I_s$		$I$		$\beta_{ss}$	
	b	$pR^2$	b	$pR^2$	b	$pR^2$
<b>Environmental</b>						
Temperature: field			-0.08	0.23		
Temperature: mean yearly					0.05	0.81
Salinity						
Secchi disk depth						
Seagrass: mean height						
Seagrass: total shoot density						
Seagrass: total biomass			0.008	0.76		
<b>Morphological</b>						
Standard length: males						
Standard length: females						
Body Depth: females					0.12	0.17
<b>Demographic</b>						
Sex ratio: adult						
Sex ratio: operational						
Population density: adult						
Population size: adult						
<b>Model <math>R^2</math></b>		0.00		0.99		0.98

estimates of  $I_s$  for males (Tables 4.7 & 4.8). The female reproductive contribution is highly correlated by field temperatures and, to a lesser extent, total seagrass biomass, accounting for 78 and 22 percent of the total variation, respectively. Male  $\bar{X}_{ms}$  loads highest for adult population size and is negatively correlated with field water temperature with a very small but significant influence of adult sex ratio. Male  $\bar{X}_{rs}$  was influenced only by the adult sex ratio which accounted for 85% of the model variation. The three Bateman's principles,  $I$ ,  $I_s$  and  $\beta_{ss}$  calculated for males also varied in the relative contributions of ecological factors. No dependent or combination of dependent variables accounted for any of the variation in  $I_s$ . Male  $I$  was highly influenced by field temperature, although the highest loading was from Secchi disk depth, negatively correlating with turbidity. Similar to mating success,  $I$  loaded highest for a temperature estimate, in this case temperature recorded in the field. Seagrass biomass and male population density also contributed significantly to  $I$ . Finally,  $\beta_{ss}$  was highly influenced by mean yearly temperature and to a morphological character, female body depth.

## Discussion

This study documents variation in the genetic mating system of the dusky pipefish from several populations throughout their distribution from the continental U.S. coast. Populations of *S. floridae* differed in adult and operational sex ratios, population density and population size and experienced dissimilar environmental regimes such as temperature, salinity, turbidity and seagrass habitat. Both males and females were demonstrated to mate multiply in all populations and the female reproductive contribution varied among different populations. Populations also differed in the numbers of mates and embryos per pregnancy and male mating system correlates such as the mean mating success, mean reproductive success, and the Bateman gradient. Results of this study also provide good evidence that mating system correlates and Bateman's principles are significantly influenced by specific ecological criteria. Both demographic and environmental factors play a major role in shaping the genetic mating system, whereas morphological factors such as body size, which are important in regulating specific mate choice behaviors in pipefishes (e.g. Berglund et al. 1988), appear not to have a major influence on the mating system over a broad geographic scale.

### *The effect of morphology on the genetic mating system*

As previously stated, morphological differences in males and females between populations appear to play a diminished role in shaping the genetic mating system in *S. floridae*. Previous investigations show a clear link between male body length and the number of embryos per brood and female body length to the number of eggs transferred

by females (Berglund et al. 1988). Larger body length in males is also correlated with an increase the mean number of mates per male within natural populations of *S. floridae* (Jones & Avise 1997b, **CHAPTER III**). Despite differences in mean body length between populations, there was no apparent effect on the genetic mating system. It may therefore be concluded that differences in male and female body depth do not explain geographic patterns of genetic mating systems, but rather play a more significant role in modulating specific mating behaviors within each population.

Female body depth does appear to play a small but significant a role in the relationship between mating and reproductive success as populations with greater than average female body depth had higher estimates of the slope of the Bateman gradient. This result is likely due to the relative increase in fecundity with female body size found in other syngnathids (Berglund et al. 1988) and a significant correlation with female body size and body depth ( $P < 0.03$ ) found in this study.

#### *The effect of abiotic environmental factors on the genetic mating system*

Of the abiotic environmental factors investigated in this study, only mean yearly water temperature and water temperature measured in the field at the time of collection explained a significant portion of the variation in several mating system estimates including the number of eggs transferred by females, male mating success, the opportunity for selection and the Bateman gradient. These results are not surprising given that water temperature plays a significant role in the potential reproductive rates of adults in congeners (Ahnesjö 1995). As water temperature increases, the potential reproductive rate, or the number of potential offspring produced by either males or

females, increases in both sexes of the broad-nosed pipefish, *S. typhle* (Ahnesjö 1995). As evidence of this relationship, populations measured that experienced lower mean yearly water temperatures such as the VA and NC sites, had fewer eggs transferred by females than sites that experienced warmer mean yearly water temperatures, conforming to expectations based on the results from *S. typhle*. Given this depression in the number of eggs transferred, it would require more females to fill a male with a full complement of eggs and therefore affect the number of mates per male and the male genetic mating system.

Mean yearly water temperature may also affect the genetic mating system in different populations as warmer water is strongly correlated with the initiation and length of breeding season in *S. floridae* (Brown 1972, Mercer 1973). Populations of *S. floridae* from the Gulf of Florida have a longer mating season than the Virginia population (Brown 1972, Mercer 1973). Similarly, temperature affects the onset and duration of the breeding season in other species of syngnathids (Ahnesjö 1995, Vincent et al. 1995, Monteiro et al. 2001, Watanabe & Wantanabe 2001, Power & Attrill 2003) and may serve to explain the high influence of this parameter on the mating system.

Other environmental factors such as salinity and turbidity investigated in this study appeared not to have any effect on the genetic mating system of *S. floridae*. Salinity likely plays a more significant role in restricting the species to saline waters as this species can tolerate a wide range of salinities but is not found in habitats lower than 10 ‰ (Dawson 1982). It was surprising that turbidity did not influence the genetic mating system since increased turbidity has recently been linked to a reduction in the

strength of sexual selection among several species of fish (Seehausen et al. 1997, Järvenpää & Kindström 2004, Candolin et al. 2007). Because all of these studies have focused on manipulating turbidity or chronicling the change in turbidity due to anthropomorphic sources, turbidity may be more influential to the genetic mating system if disturbed rather than the norm experienced in each population.

*The effect of habitat on the genetic mating system*

Many species of pipefishes are attracted to seasonal seagrass beds during the breeding season to find mates and likely rely on seasonal seagrass beds for protection from predation and to serve as a nursery for young (Vincent et al. 1995, Wantanabe & Wantanabe 2001). Theoretically, the type and density of habitat may be important in structuring the genetic mating systems of vertebrates (Weatherhead & Robertson 1997, Turner & McCarty 1998). In this study, there is a positive relationship between mean shoot height and both mean yearly temperature ( $P < 0.003$ ) and salinity ( $P < 0.03$ ), but no correlation with mating system parameters was detected. Similarly, total shoot density was not positively correlated with any genetic mating system criteria. However, there was a significant effect of total aboveground seagrass biomass on both the opportunity for selection and the female reproductive contribution. This result is interesting since it is not clear how a causal relationship between the genetic mating system and seagrass biomass could exist and thus may be a spurious result. For example, seagrass biomass is correlated with species of seagrass, levels of ambient light, nutrient availability and grazing levels (Estes & Peterson 2000, Heck & Valentine 2006),



none of which were investigated in this study. Seagrass biomass, on the other hand, may influence the genetic mating system by governing species interactions because this measure was significantly correlated with two demographic factors; the operational sex ratio ( $P < 0.007$ ) and the total adult population size ( $P < 0.05$ ).

#### *Demographic influences on the genetic mating system*

Adult sex ratios explained a large portion of the variation in male mating and reproductive success experienced between populations. This result is driven, in part by the high mean male mating success experienced by the two populations that had a male-biased sex ratio (VA and TX). This result is interesting because these two populations had large differences in the number of mates per pregnancy and body length of males. The high number of unmated males in the Texas population and the low number of unmated males in the VA population cast a large influence on the mating success of males and may help to explain the similarities between sites in terms of the ASR and hence the male mating success.

Adult population size was a major influence on the mean mating success between populations. A larger population size may result in more mate encounters for individuals and may increase the chance of finding suitable mates. This result should be viewed with caution however, since adult population size is strongly correlated with mean yearly temperature and mean mating success may be directly related to temperature-dependant female potential reproductive rates encountered in other pipefish species (Ahnesjö 1995).

The fact that the operational sex ratio did not explain a significant amount of the variation in any mating system parameter in this study is remarkable, given the emphasis placed on the operational sex ratio as the prime mating system determinant (i.e., Kvarnemo & Ahnesjö 1996). However, because the operational sex ratio can fluctuate over the course of the mating period, estimates taken at the time of collection may not represent the ecological conditions experienced by individuals during mating. Therefore, the influence of the operational sex ratio cannot be definitively ruled out as a major influence on the genetic mating system (see **CHAPTER V**).

### *Conclusions*

This study is the first to attempt to link genetic mating system correlates to specific ecological variables in a systematic manner on a broad geographic scale. Although ecological variables selected in this model are specific to this particular system, it highlights the importance of investigating ecological factors in a systematic and comprehensive fashion to make accurate predictions concerning mating system evolution. Assessment of the multiple criteria such as demographic and environmental factors that likely play an important role of shaping and defining the genetic mating system is thus critical to our understanding of how genetic mating systems are organized and evolve.

**CHAPTER V**  
**TEMPORAL VARIATION IN THE MATING SYSTEM OF THE**  
**BROAD-NOSED PIPEFISH, *SYNGNATHUS TYPHLE***

**Introduction**

A fundamental determinant of mating system organization is the availability and spatial distribution of mates (Emlen & Oring 1977, Shuster & Wade 2003). In species with conventional sex roles, males compete with one another for access to females. The degree to which females are spatially distributed may affect the ability of a male to monopolize mates. For instance, if females are crowded around a particular resource, the ability of one male to monopolize a larger proportion of females increases because of his close proximity to several females (Emlen & Oring 1977). In addition to spatial dispersion of females, temporal patterns of female receptivity may also affect a male's opportunity to monopolize access to mates (Emlen & Oring 1977). If all females become receptive at one particular time, then a male's ability to monopolize many females simultaneously is reduced. Hence, the spatial dispersion of mates and the degree of mating synchrony play important roles in determining the intensity of sexual selection (Shuster & Wade 2003).

In light of the temporal and spatial dispersions of available mates, one particularly enlightening mating system variable is the operational sex ratio (OSR, Emlen & Oring 1977). The OSR is the ratio of potentially breeding males to all potentially breeding adults in the population (i.e., it is the proportion of receptive adults that are male). Because the proportion of potential breeders of each sex determines the

OSR, it is related to the relative variances in mating success of the two sexes.

Departures from an OSR of 0.5 signify a sex-biased situation in which competition for mates is expected to be stronger in the more abundant sex, thus increasing the environmental potential for polygamy (Emlen & Oring 1977, Kvarnemo & Ahnesjö 1996, Shuster & Wade 2003). Of course, increased competition should favor the evolution of traits that increase the chances of their bearers to find and monopolize mates (Shuster & Wade 2003).

Many species undergo dramatic shifts in the OSR during a breeding season. Examples include such species as bushcrickets (Simmons 1992), fishes (Vincent et al. 1994, Kvarnemo 1996, Oliveira et al. 1999, Forsgren et al. 2004), anurans (Lodé et al. 2005), and birds (Johnson et al. 2002). Competition for resources, such as high quality food, territories, nesting sites and mates, can dramatically change the OSR for a particular sex during the course of the breeding season (Simmons 1992, Vincent et al. 1995, Forsgren et al. 2004). However, it is unclear what effect a fluctuating OSR may have on the genetic mating system, fitness components of individuals, or selection on particular sexually selected traits. Thus far, insufficient empirical work has been done to allow accurate predictions of the outcome of such a scenario. Therefore the detailed genetic study of natural shifts in the OSR may be a fruitful approach to the development of a more complete theory of sexual selection.

The purpose of this study is to investigate the extent to which a changing OSR influences the genetic mating system in the broad-nosed pipefish, *Syngnathus typhle*. The questions posed during the study were: (1) does an increasingly female-biased OSR

affect the genetic mating system? And (2) do measures of the genetic mating system correctly predict and respond to changes in hypothesized mate competition as measured by the OSR?

The broad-nosed pipefish is well suited to this type of inquiry because the OSR changes substantially during the course of a relatively short breeding season (Vincent et al. 1994, Vincent et al. 1995). Both male and female *S. typhle* invade seagrass beds in early May and commence mating when water temperatures reach approximately 16°C (Vincent et al. 1994, Vincent et al. 1995, Ahnesjö 1995). Upon the arrival of the fish into the shallow seagrass, at the commencement of mating, the OSR is close to 0.5 (i.e., equal number of receptive males and females; Berglund et al. 1986, Vincent et al. 1994). During mating, females transfer unfertilized eggs to a pouch on the male's ventral surface and the male fertilizes the eggs within the pouch. Hence, male *S. typhle* provide all parental care to embryos, and the "pregnant" males are unable to mate until they give birth to their progeny. A typical male receives eggs from multiple females per pregnancy, and the process of filling the brood pouch occurs over a time period of a few days at most (Berglund et al. 1988). Once males receive a full complement of eggs, the brood pouch seals. Males give birth four to six weeks later, depending on water temperature (Berglund et al. 1989, Ahnesjö 1995). Because the receptivity of males is constrained by the length of male pregnancy, an excess of females appears and grows as the breeding season progresses, resulting in an increase in female competition for males and a decrease in the operational sex ratio (Vincent et al. 1994). The OSR is consequently female-biased and female-female competition for mates is predicted to

increase (Vincent et al. 1994; Vincent et al. 1995; Kvarnemo & Ahnesjö 1996, Shuster & Wade 2003). Because the length of pregnancy varies among males, they become receptive after their first pregnancy in an asynchronous fashion (Vincent et al. 1994). Consequently, we would predict that this increase in competition to mate with males among females likely has a dramatic affect on the direction and intensity of sexual selection as the mating season progresses.

## **Materials and Methods**

### *Sample collection*

The study was conducted on the west coast of Sweden during the summers of 2005 and 2006. Five sites (Table 5.1, Fig. 5.1) were visited prior to and during the breeding season in the summer of 2005 and two sites were revisited in 2006. During each visit, adult *S. typhle* were collected from shallow (1-6m) eelgrass (*Zostera marina*) beds by using a beam trawl with a 2mm mesh towed from a motorized boat. Each site was trawled for approximately two hours (8-10 trawls) during each visit. Once caught, males and females were separated into buckets containing seawater. Each individual was measured for standard length to the nearest mm (SL; tip of rostrum to the base of the caudal peduncle). Additionally, males were assessed for pregnancy status including presence of eggs, proportion of brood pouch filled with eggs and stage of development of embryos. After all measurements were taken individuals were released back to their corresponding collection sites.

Table 5.1. GPS coordinates for Swedish sampling sites. Letters correspond to locations in Fig. 5.1.

	<b>Site</b>	<b>Coordinates</b>
<b>A</b>	Trinnhålet	N58°14.441', E11°22.849'
<b>B</b>	New Galveston	N58°14.314', E11°23.079'
<b>C</b>	Bökevik	N58°14.926', E11°26.709'
<b>D</b>	Kvarnbukten	N58°15.627', E11°28.381'
<b>E</b>	Tjuvsund	N58°15.820', E11°29.753'

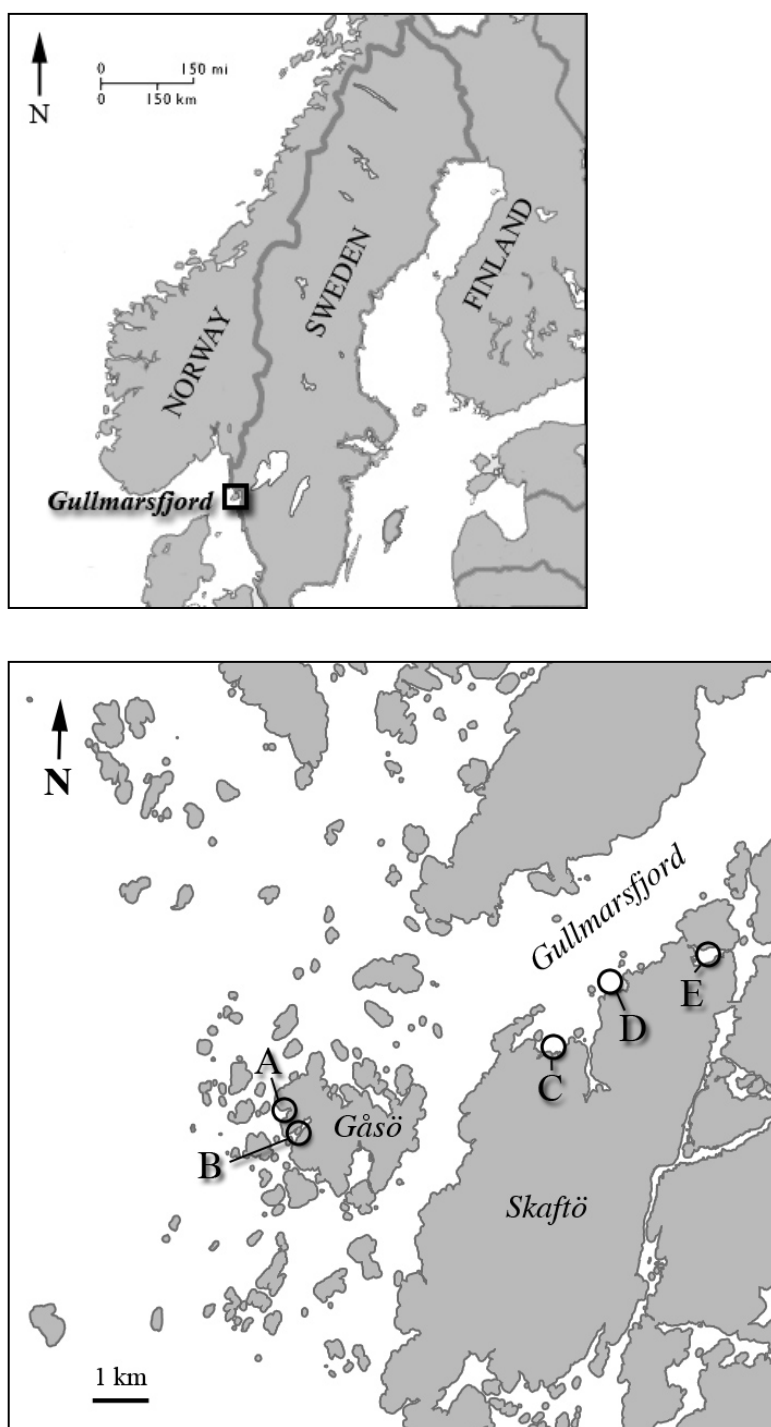


Figure 5.1. Collection sites on the Swedish west coast. A = Trinnhålet, B = New Galveston, C = Bökevik, D = Kvarnbukten, E = Tjuvsund.



Adult sex ratios (ASR) and operational sex ratios (OSR) were calculated for all site visits. The ASR is the number of adult males divided by the total number of adults collected and the OSR is equivalent to the ratio of receptive males to the total number of receptive adults. Because males may take several days to fill their brood pouches, receptive males included those males that could still accept eggs in their brood pouch as evidenced by space in the top of the brood pouch and an open (non-sealed) brood pouch (Vincent et al. 1994). The ASR and OSR were tested at each sampling time for departures from equality with a  $\chi^2$  test.

The Trinnhålet site was chosen for an intensive capture-mark-recapture study to estimate population size, to gauge the progression of the breeding cycle and to measure physical attributes of potential mates during the 2005 breeding season. The site was visited twice a week and adult males and females were collected and measured. In addition, a small fin clip from the caudal fin was taken from each adult and preserved in 95% EtOH. Recaptured individuals were remeasured and fin clipped before returning them to the site. Population size based on fin clips was calculated using the Lincoln-Peterson capture-mark-recapture method (Pollock et al. 1990).

Prior to the birth of the earliest broods of the first pregnancy in 2005, the Trinnhålet and New Galveston sites were visited on several days (6/16-6/26) to harvest pregnant males for parentage analysis and to estimate population size. Similarly, the Trinnhålet, New Galveston and Tjuvsund sites were visited during the second pregnancy of 2005 (7/16-7/28) and Tjuvsund and Trinnhålet sites were revisited during the first pregnancy in 2006 (6/15-6/19). During these visits all adults were measured and fin

clipped, and all pregnant males were harvested. Pregnant males were transported to Kristineberg Marine Research Center and sacrificed by severing their spinal column anterior to the operculum. Whole fish, including the embryos, were preserved in 95% ethyl alcohol for parental analysis. Fin clips taken from all adults (males and females) during these collection episodes were genotyped and the genotypes were used to match sampled adult females to the genotypes of the mates of pregnant males that we constructed using the genotypes of embryos. These data were used to estimate female population size using the modified Lincoln-Peterson capture-mark-recapture method for parentage studies (Jones & Avise 1997b). Marks for pregnant males removed for parentage analysis that were marked earlier in the study were deducted from the total number of marks for subsequent sampling times.

#### *Genetic analysis*

A Gentra PureGene™ cell and tissue kit was used to extract DNA from adult fin tissue. Each adult was genotyped using four polymorphic microsatellite loci (*Typh04*, *Typh12*, *Typh16*, *Typh18*) previously employed in *S. typhle* (Jones et al. 1999). Brood pouches of pregnant males were dissected, and all developed embryos were plucked from the male brood pouch with flame-sterilized forceps after noting the relative position of each embryo and any abnormal or undeveloped embryos. Developed embryos were then digested using a 5% Chelex/Protenase K digestion in a 96 well plate (Miller & Kapuschinski 1996). Three microsatellite loci, *Typh04*, *Typh16* and *Typh18* were used to genotype all embryos and to assign parentage to offspring (Jones et al.

1999). Every fourth embryo was amplified using PCR conditions outlined by Jones et al. (1999). Maternal genotypes were reconstructed following the protocols outlined in the **Methods** section of **CHAPTER III**. When a reconstructed maternal genotype was only supported in one embryo, additional embryos surrounding that physical location in the brood pouch were genotyped. All microsatellite fragment analyses were performed on an ABI Prism® 3730 DNA Analyzer and resulting fragments were scored using ABI Prism® GeneMapper™ software (Applied Biosystems, Foster City, CA).

#### *Quantification of the genetic mating system by Bateman's principles*

Several measures of the genetic mating system, including the mean mating success ( $\bar{X}_{ms}$ ), mean reproductive success ( $\bar{X}_{rs}$ ), the index for resource monopolization ( $Q_{ms}$ ,  $Q_{rs}$ ), the Morisita index ( $I_{\delta-ms}$ ,  $I_{\delta-rs}$ ), the opportunity for selection ( $I$ ), the opportunity for sexual selection ( $I_s$ ), and the Bateman gradient ( $\beta_{ss}$ ), were calculated for males following formulas presented in the **CHAPTER I** and the **Methods** section of **CHAPTER III**.

#### *Statistical analysis*

All statistics were analyzed first for normality and equal variances. If these assumptions were not met, data were transformed or if no transformation satisfied *a priori* assumptions, appropriate non-parametric tests were applied. Statistical tests as well as any transformations are indicated throughout the text. All statistical analyses were performed with JMP IN™ statistical software package version 5.1 (SAS Institute

Inc. Cary NC). Means are reported throughout the text  $\pm$  the standard error of the mean ( $\pm SE$ ).

## Results

### *The ASR and OSR*

In all sites visited during the 2005 and 2006 field seasons, the ASR indicated either equal numbers of males and females or a significant excess of males (Table 5.2 & 5.3, Fig. 5.2). The Tjuvsund site was found to have a highly significant male-biased ASR in both years as few females were encountered during these times. The OSR, on the other hand, underwent a predictable decrease toward an excess of females during the 2005 sampling year, mirroring the pattern previously recorded for this species (Vincent et al. 1995). The OSR reached an extreme female-bias during the 1<sup>st</sup> pregnancy except in the Tjuvsund site where the OSR was found to fluctuate from male-biased to female-biased on different sampling dates (Tables 5.2 & 5.3, Fig. 5.3). As males gave birth and became available for additional matings, the extreme excess of females disappeared (Fig. 5.3).

### *Mark-recapture data*

Capture-mark-recapture data from the Trinnhålet focal site showed a relatively small adult population size when compared to population size values in Tjuvsund during 2005 and 2006 (Fig. 5.2 & 5.3). The New Galveston site yielded a similar adult population size to Trinnhålet (Fig. 5.2 & 5.3). The large increase in the population size in Trinnhålet after the first pregnancy (7/3,7/8) is likely an overestimate

Table 5.2. Data from 2005 and 2006 sites. Sample sizes of total adults ( $n$ ), males ( $m$ ), non-pregnant males ( $m'$ ) and females ( $f$ ) are listed and adult sex ratios (ASR) and operational sex ratios (OSR) are calculated for each sampling time. Population size is estimated in all sites except Bøkevik and Kvarnbukten using the Peterson-Lincoln capture-mark-recapture method. Asterix denote significant departures from equality ( $\chi^2$  test P value  $* < 0.05$ ,  $** < 0.01$ ,  $*** < 0.001$ ).

Date	Sample Size				ASR	OSR	$n_m$	$n_r$	Population size ( $\pm$ 95% CI)
	$n$	$m$	$m'$	$f$	$m/n$	$m'/(f+m')$			
2005									
Bökevik									
5/13	46	24	21	22	0.52	0.49	---	---	---
5/17	24	13	10	11	0.54	0.48	---	---	---
5/19	22	15	14	7	0.68	0.67	---	---	---
5/30	107	59	29	48	0.55*	0.38	---	---	---
6/01	24	15	0	9	0.63*	0.00	---	---	---
Kvarnbukten									
5/12	35	18	18	17	0.51	0.51	---	---	---
5/14	52	35	35	17	0.67**	0.67**	---	---	---
6/02	37	21	2	16	0.57	0.11*	---	---	---
6/04	19	11	1	8	0.58	0.11**	---	---	---
6/28	17	13	3	4	0.76*	0.43	---	---	---
7/11	21	13	1	8	0.62	0.11**	---	---	---
New Galveston									
6/17	26	12	0	14	0.46	0.00**	---	---	---
6/18	31	16	0	15	0.52	0.00**	---	---	---
7/25	18	16	6	2	0.89*	0.75	14	---	---
7/27	25	15	8	10	0.60	0.44	---	1	350 (153-547)
Tjuvsund									
7/20	46	31	13	15	0.67**	0.46	34	---	---
7/21	43	22	9	21	0.51	0.30*	25	0	---
7/22	21	17	11	4	0.81*	0.73*	13	2	620 (229–1010)
7/24	36	27	16	9	0.75**	0.64	---	2	1296 (468-2124)
2006									
Trinnhålet									
6/19	32	18	1	14	0.56	0.07*	18	---	---
6/20	62	37	2	25	0.60	0.07***	---	10	112 (75-148)
Tjuvsund									
6/15	138	120	34	18	0.87***	0.65*	118	---	---
6/16	99	89	24	10	0.90***	0.71	---	3	3894 (1382-6406)

Table 5.3. Detailed study of Trinnhålet Bay 2005. Sample sizes of total adults ( $n$ ), males ( $m$ ), non-pregnant males ( $m'$ ) and females ( $f$ ) are listed and adult sex ratios (ASR) and operational sex ratios (OSR) are calculated for each sampling time. Each adult was finclipped when captured and the total numbers of individuals marked ( $n_m$ ) and recaptured ( $n_r$ ) are listed. Dates in bold represent times when pregnant males were harvested from the sites. Marked pregnant males that were removed from the site are subtracted from the total of marked individuals (data not shown). Population size is estimated using the Peterson-Lincoln capture-mark-recapture method. Asterix denote significant departures from equality ( $\chi^2$  test P value\* < 0.05, \*\* < 0.01, \*\*\* < 0.001).

Date	Sample Size				ASR	OSR	$n_m$	$n_r$	Population size ( $\pm$ 95% CI)
	$n$	$m$	$m'$	$f$	$m/n$	$m'/(f+m')$			
5/12	39	23	20	16	0.59	0.56	19	---	---
5/16	29	15	14	14	0.52	0.50	40	1	551 (240-862)
5/20	17	13	9	4	0.76	0.69	54	3	227 (91-362)
5/24	24	15	7	9	0.63	0.44	70	8	162 (93-231)
6/03	27	16	5	11	0.59	0.31	84	11	185 (116-253)
6/12	47	26	0	21	0.55	0.00***	110	16	247 (167-326)
<b>6/16</b>	18	6	0	12	0.33	0.00**	113	9	220 (138-302)
<b>6/17</b>	29	12	1	17	0.41	0.06**	123	14	234 (160-308)
<b>6/21</b>	8	2	0	6	0.25	0.00*	125	3	328 (149-507)
<b>6/26</b>	11	8	1	3	0.73	0.25	127	2	250 (121-379)
7/03	10	9	5	1	0.90	0.83	136	1	1397 (610-2184)
7/08	7	4	3	3	0.57	0.50	142	1	952 (419-1485)
7/12	5	4	3	1	0.80	0.75	145	2	355 (159-551)
<b>7/18</b>	10	5	1	5	0.50	0.17	148	6	241 (148-335)
<b>7/19</b>	5	3	0	2	0.60	0.00	146	3	246 (135-358)
<b>7/21</b>	6	4	2	2	0.67	0.50	149	3	292 (146-438)
<b>7/28</b>	6	4	2	2	0.67	0.50	---	4	223 (135-312)
<b>Total</b>							<b>149</b>	<b>87</b>	<b>436 (390-484)</b>

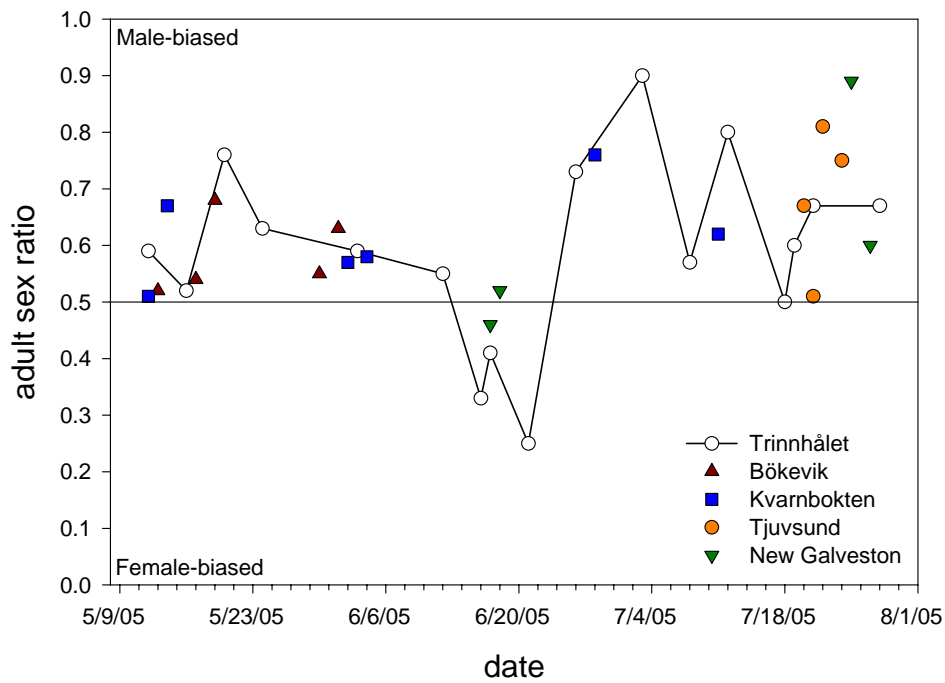


Figure 5.2. Adult sex ratio (number of males/divided by total number of adults) recorded for the 2005 sampling locals. The line at 0.5 denotes equal numbers of males and females captured.

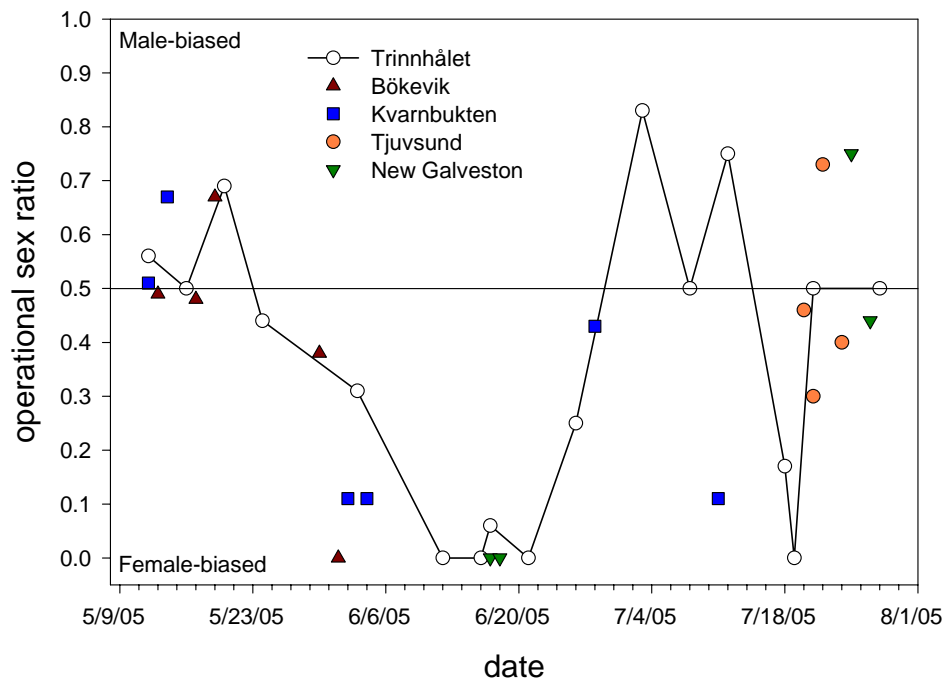


Figure 5.3. The operational sex ratio (proportion of males available for mating divided by the total number of adults available for mating) for the 2005 sampling locals. A solid line denotes an equal OSR at 0.5. Competition for males among females should be highest when the OSR is lowest.



since this increase coincided with the removal of marked males from the site and may represent new migration into the site. Genetic analysis of fin clips revealed multiple recaptures of the same adults in the in all sites and years. Some individuals were recaptured up to five times in the Trinnhålet site during the 2005 intensive sampling. Genetic analysis failed to record migration between sites and no individuals were recaptured in 2006 that were fin clipped in 2005.

#### *Analysis of pregnant males*

The pregnant males subjected to parentage analysis were analyzed with respect to the number of mates per pregnancy, the number of embryos per pregnancy and body length (Table 5.4). All sites and times were used except the collections made in New Galveston and Trinnhålet during the 2<sup>nd</sup> pregnancy due to low numbers of pregnant males collected during these times (Table 5.4). There were no significant differences between sites in the number of mates per mated males (ANOVA  $F_{1,129} = 2.39$ ,  $P = 0.054$ ) and the number of embryos per mated male among sites and times (ANOVA  $F_{1,129} = 1.44$ ,  $P = 0.23$ ). A significant difference in body length (SL) was encountered between sites (Kruskal-Wallis Rank Sums Test:  $\chi^2_{1,129} = 24.30$ ,  $P < 0.0001$ ). Captured pregnant males were significantly larger in the Trinnhålet site in both years than in the Tjuvsund site in 2005. Pregnant males also were larger in the Trinnhålet site in 2005 than in the Tjuvsund site in 2006. This difference in male standard length did not alter the relationship between number of embryos and standard length (Site\*SL ANCOVA:  $F_{1,124} = 0.69$ ,  $P = 0.60$ ), and all males had a similar positive relationship between

Table 5.4. Summary statistics for pregnant males analyzed for parentage. Site, date and time (1 = 1<sup>st</sup> pregnancy, 2 = 2<sup>nd</sup> pregnancy), number of males analyzed ( $n_m$ ), number of mates (mates), number of embryos (embryos) and standard length (SL) are listed. Asterix denote sites/times not included in final analyses due to small sampling sizes ( $n$ ) encountered during these times.

Year	Site	Date	Time	$n_m$	Mates ( $\pm$ SE)	Embryos ( $\pm$ SE)	SL (mm $\pm$ SE)
2005	Trinnhålet	6/16-6/26	1	27	3.44 $\pm$ 0.27	73.4 $\pm$ 5.3	194.0 $\pm$ 5.2
	New Galveston	6/17-6/18	1	20	3.80 $\pm$ 0.32	69.1 $\pm$ 6.1	177.9 $\pm$ 6.1
	Trinnhålet*	7/18-7/28	2	6	2.33 $\pm$ 0.59	108.3 $\pm$ 11.2	201.0 $\pm$ 11.1
	New Galveston*	7/25-7/27	2	2	3.00 $\pm$ 1.01	78.5 $\pm$ 19.4	153.5 $\pm$ 19.1
	Tjuvsund	7/20-7/24	2	27	3.33 $\pm$ 0.28	61.9 $\pm$ 5.3	157.6 $\pm$ 5.2
2006	Trinnhålet	6/19-6/20	1	30	3.87 $\pm$ 0.26	79.0 $\pm$ 5.0	178.5 $\pm$ 4.9
	Tjuvsund	6/15-6/16	1	30	4.40 $\pm$ 0.26	71.6 $\pm$ 5.0	167.4 $\pm$ 4.9

standard length and number of embryos (Site ANCOVA:  $F_{1,128} = 0.91$ ,  $P = 0.46$ ; SL ANCOVA:  $F_{1,128} = 11.69$ ,  $P = 0.0008$ ). However significant differences in the number of mates as a function of SL was detected among sites (site ANCOVA:  $F_{1,128} = 3.59$ ,  $P = 0.0083$ ) and this relationship was positive (SL ANCOVA:  $F_{1,128} = 14.41$ ,  $P = 0.0002$ ). The Trinnhålet site had significantly more mates per unit of SL than all other sites and the 2005 Tjuvsund site had significantly fewer mates per unit of SL than all other sites (SL ANOVA  $F_{4,128} = 3.59$ ,  $P = 0.0083$ ). The slope of the relationship between number of mates and standard length was not significantly different between sites (SL\*Site ANCOVA:  $F_{1,124} = 1.10$ ,  $P = 0.36$ ).

#### *Quantification of the male genetic mating system*

Despite similarities among the number of mates and embryos per mated male, significant differences in mating system estimates were apparent. Mean male mating success ( $\bar{X}_{ms}$ ) was significantly lower in the 2<sup>nd</sup> pregnancy in the Tjuvsund site than all the 1<sup>st</sup> pregnancy collections except for the 2006 Trinnhålet collection (ANOVA  $F_{4,153} = 3.76$ ,  $P = 0.006$ ; Tukey-Kramer post-hoc test,  $\alpha = 0.05$ ; Table 5.5). This low estimate of  $\bar{X}_{ms}$  in the 2<sup>nd</sup> pregnancy collection is due to the high proportion of unmated males encountered during the sampling period. Estimates of  $I_s$ ,  $Q_{ms}$ , and  $I_{\delta-ms}$  were highest for the 2005 2<sup>nd</sup> pregnancy at Tjuvsund relative to all other samples, although estimates of  $I_s$  were not significantly different among sites or sampling times (Levene Homogeneity of Variance Test:  $F_{4,153} = 1.72$ ,  $P = 0.1488$ ).

In a similar fashion, estimates of  $\bar{X}_{rs}$  were lowest in the 2<sup>nd</sup> pregnancy sample for Tjuvsund during 2005 ( $F_{4,153} = 5.27$ ,  $P = 0.0005$ ) and significantly different from all other sites except the 2006 Trinnhålet collection (Tukey-Kramer post-hoc test,  $\alpha = 0.05$ ). This difference was mirrored in the standardized variance in reproductive success,  $I$  (shown here as the relative fitness of reproductive success), showing a significant increase in the variance in 2005 Tjuvsund and 2006 Trinnhålet site (Levene Homogeneity of Variance Test:  $F_{4,153} = 6.99$ ,  $P < 0.0001$ , Table 5.5). Estimates of  $Q_{rs}$  and  $I_{\delta-rs}$  were also highest for the 2005 Tjuvsund sample (Table 5.5).

The relationship between mating and reproductive success as measured by the Bateman gradient ( $\beta_{ss}$ ) was significantly different among sites and sampling times (Site\*Mates ANCOVA:  $F_{9,157} = 7.73$ ,  $P < 0.0001$ , Fig. 5.4). The 2<sup>nd</sup> pregnancy had a significantly larger estimate of  $\beta_{ss}$  than all 1<sup>st</sup> pregnancy sites surveyed (Table 5.5, Fig. 5.4). The smallest estimate of  $\beta_{ss}$  was recorded for the 2006 Trinnhålet site during 2006. This estimate was significantly smaller than the Trinnhålet 2005 collection and both Tjuvsund site collections but not the New Galveston site collection.

#### *Female mating behavior*

Females displayed morphological differences among sites during the 2005 and 2006 sampling years. The largest females were encountered in the Trinnhålet and New Galveston sites during 2005 (Table 5.6). These females were of similar size to those collected from Trinnhålet in 2006 but were larger than females encountered in both

Table 5.5. Quantitative characterization of mating system estimates for the 2005-2006 collection sites. Shown estimates are mean mating success ( $\bar{X}_{ms}$ ), the variance in mating success ( $\sigma_{ms}$ ), the opportunity for sexual selection ( $I_s$ ), the index of resource monopolization for mating success ( $Q_{ms}$ ), the Morisita index for mating success ( $I_{\delta-ms}$ ), mean reproductive success ( $\bar{X}_{rs}$ ), the variance in reproductive success ( $\sigma_{rs}$ ), the opportunity for selection ( $I$ ), the index of resource monopolization for reproductive success ( $Q_{rs}$ ), the Morisita index for reproductive success ( $I_{\delta-rs}$ ) and the Bateman gradient ( $\beta_{ss} \pm SE$ ).

Date	Site	Time	<i>Mating Success</i>					<i>Reproductive Success</i>					
			$\bar{X}_{ms}$	$\sigma_{ms}$	$I_s$	$Q_{ms}$	$I_{\delta-ms}$	$\bar{X}_{rs}$	$\sigma_{rs}$	$I$	$Q_{rs}$	$I_{\delta-rs}$	$\beta_{ss} (\pm SE)$
2005	Trinnhålet	1	3.32 $\pm$ 0.35	2.3002	0.21	-0.0047	0.91	70.8 $\pm$ 6.4	1217.1	0.24	0.0083	1.22	0.22 (0.05)
	New Galveston	1	3.80 $\pm$ 0.41	1.9579	0.14	-0.0058	1.10	69.1 $\pm$ 7.6	903.0	0.19	0.0059	1.44	0.18 (0.06)
	Tjuvsund	2	2.25 $\pm$ 0.29	3.1154	0.62	0.0154	1.17	41.8 $\pm$ 5.4	1747.2	0.69	0.0500	1.65	0.38 (0.05)
2006	Trinnhålet	1	3.62 $\pm$ 0.33	4.5645	0.35	0.0026	1.06	74.1 $\pm$ 6.0	912.3	0.17	0.0041	1.15	0.08 (0.03)
	Tjuvsund	1	3.47 $\pm$ 0.30	4.5804	0.38	0.0032	0.88	56.5 $\pm$ 5.5	1492.9	0.47	0.0115	1.17	0.23 (0.04)

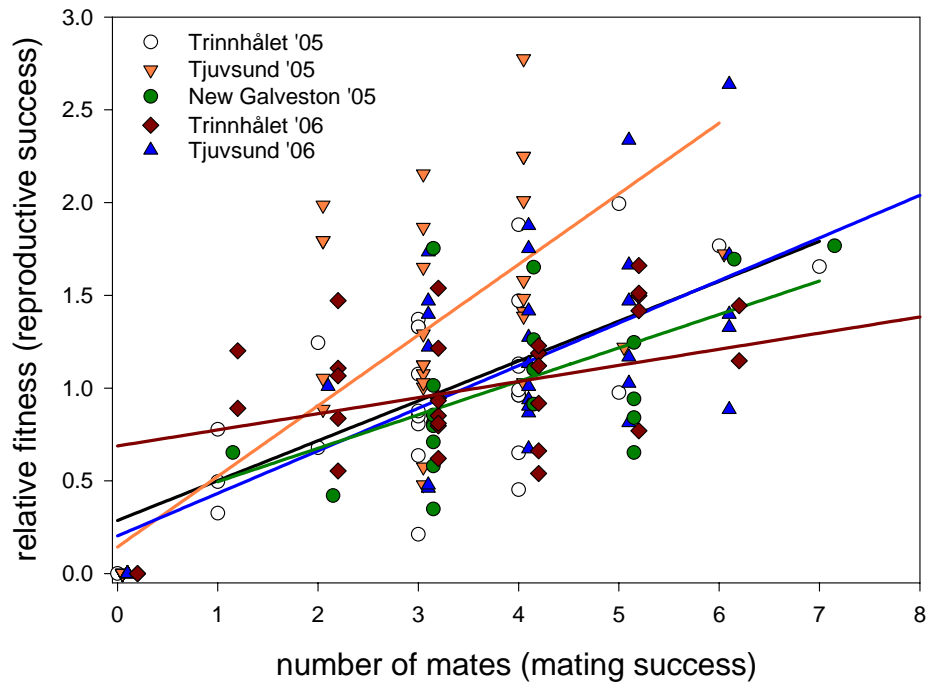


Figure 5.4. Relationship between reproductive success and mating success for males from different sites and sampling times. Bateman gradients ( $\beta_{ss}$ ) are shown as colored lines corresponding to the symbol color for each site/time. Reproductive success is shown as relative fitness, i.e., number of offspring produced divided by the mean number of offspring produced.

Table 5.6. Summary statistics for females caught during the 2005 and 2006 sampling times. Number of unique females caught ( $f$ ), standard length (SL, mm  $\pm$  SE), number of unique female reconstructed genotypes ( $f_c$ ), number of reconstructed female genotypes matched to individuals caught in the field ( $f_r$ ), percent recaptured ( $\%_r$ ), probability of identity for females caught ( $P_{ID-f}$ ), the probability of identity for reconstructed genotypes ( $P_{ID-fr}$ ) and the female population size ( $\pm$  95% CI) calculated using the modified Lincoln-Peterson method for genetic mark-recapture (Jones et al. 1997b) are listed.

Date	Site	Time	$f$	SL (mm $\pm$ SE)	$f_c$	$f_r$	$\%_r$	$P_{ID-f}$	$P_{ID-fr}$	Female population size ( $\pm$ 95% CI)
2005	Trinnhålet	1	90	209.7 $\pm$ 5.9	77	23	26	4.6 X 10 <sup>-7</sup>	3.2 X 10 <sup>-7</sup>	295 (212-378)
	New Galveston	1	29	213.4 $\pm$ 6.4	73	0	0	3.1 X 10 <sup>-6</sup>	1.2 X 10 <sup>-4</sup>	---
	Tjuvsund	2	47	183.3 $\pm$ 5.8	84	6	13	1.5 X 10 <sup>-6</sup>	3.2 X 10 <sup>-7</sup>	582 (224-939)
2006	Trinnhålet	1	37	190.2 $\pm$ 5.8	85	17	46	8.3 X 10 <sup>-6</sup>	2.9 X 10 <sup>-7</sup>	181 (128-233)
	Tjuvsund	1	28	159.4 $\pm$ 6.5	128	0	0	7.5 X 10 <sup>-7</sup>	3.8 X 10 <sup>-7</sup>	---

Tjuvsund collections, where females were significantly smaller on average (ANOVA  $F_{4,156} 11.90$ ,  $P < 0.0001$ , Kruskal-Wallis post hoc test  $\alpha = 0.05$ ).

Reconstructed female genotypes had low probabilities of identity (Table 5.6) and were therefore matched to females caught in the field with high certainty. A high proportion of females fin clips with identical three locus genotypes were encountered in the Trinnhålet site in both years (Table 5.6), indicating a high rate of recapture of females. As a result of this high recapture rate, female population size calculated with the modified Lincoln-Peterson capture-mark-recapture method (Jones & Avise 1997b) was small compared to that of the Tjuvsund 2005 sample. Estimates of female population size based on genetic parentage data for all samples agreed with physical capture-mark-recapture population sizes taken during these times (Tables 5.2, 5.3 & 5.6). In the New Galveston and Tjuvsund 2006 collections, no female genotypes reconstructed from progeny arrays were matched to actual fin-clipped females, so female population size could not be estimated for these samples.

In all samples, females mated with multiple males and samples with high recapture rates revealed high levels of polyandry (Table 5.7). The number of eggs transferred per female to mated males, also known as the female reproductive contribution (FRC), was highest among the Trinnhålet and New Galveston 2005 collections and lowest in the Tjuvsund 2006 site (Table 5.6). However, the FRC estimates were not significantly different between sites (ANOVA  $F_{4,502} = 2.30$ ,  $P = 0.0578$ ).



Table 5.7. Summary statistics for female mating behavior. Number of mates for individual females constructed from recaptured females, number of identical female reconstructed genotypes matching reconstructed genotypes (in parentheses), and the mean female reproductive contribution per mated male ( $\text{FRC} \pm \text{SE}$ ) are listed.

Date	Site	Time	Mates					FRC ( $\pm \text{SE}$ )
			1	2	3	4	5	
2005	Trinnhålet	1	14	6(2)	3(1)			$21.3 \pm 1.6$
	New Galveston	1		(3)				$18.2 \pm 1.6$
	Tjuvsund	2	5	1(3)	1			$18.7 \pm 1.9$
2006	Trinnhålet	1	10	2(8)	2(3)	2	1	$21.1 \pm 1.6$
	Tjuvsund	1		(6)				$16.1 \pm 1.2$

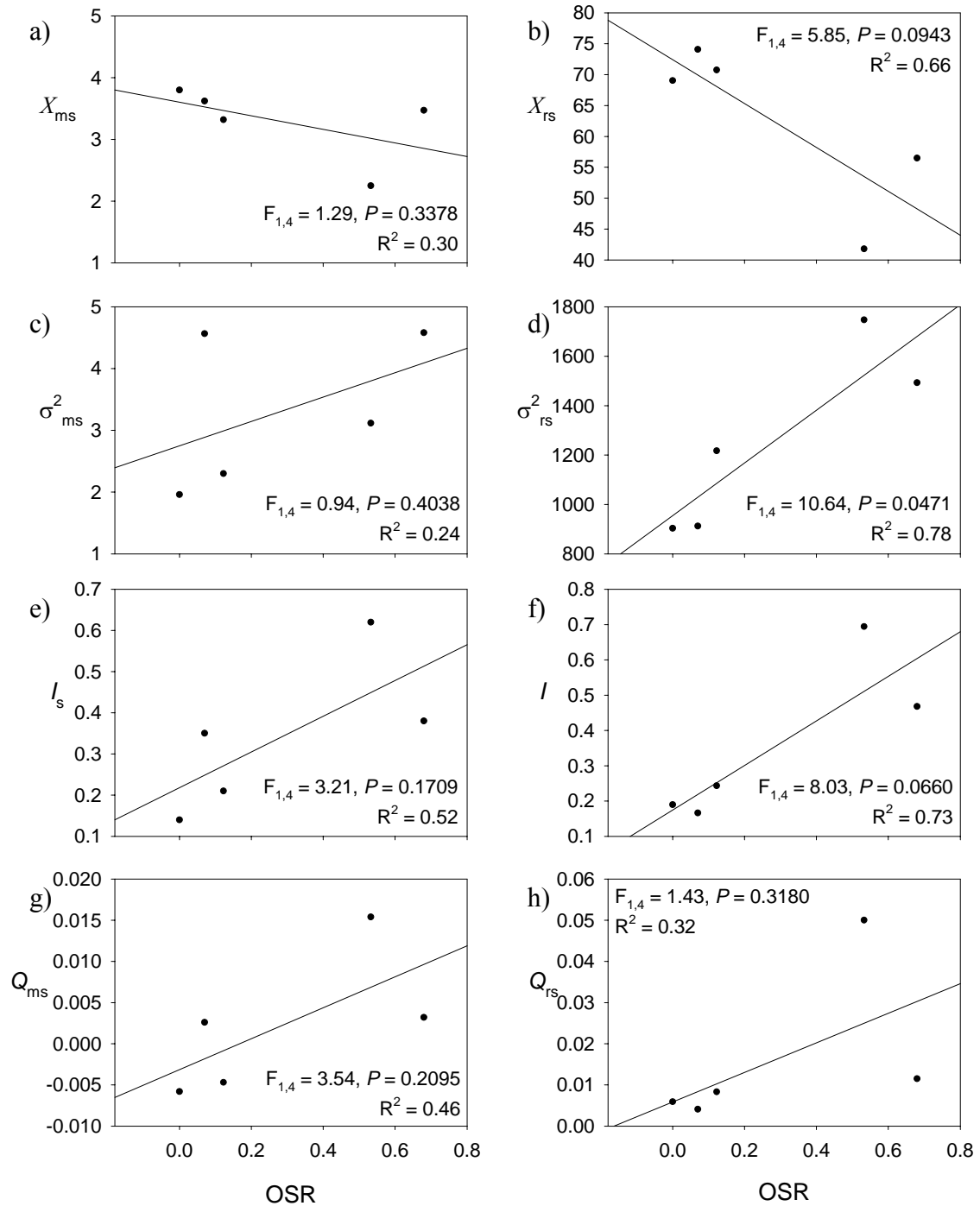


Figure 5.5. Relationship of the OSR at the time of collection with various male mating system estimates:

a) mean mating success ( $\bar{X}_{ms}$ ), b) mean reproductive success ( $\bar{X}_{rs}$ ), c) variance in mating success ( $\sigma_{ms}^2$ ), d) variance in reproductive success ( $\sigma_{rs}^2$ ), e) the opportunity for sexual selection ( $I_s$ ), f) the opportunity for selection ( $I$ ), g) the index for resource monopolization for mating success ( $Q_{ms}$ ), h) the index for resource monopolization for reproductive success ( $Q_{rs}$ ), i) the Morisita index for mating success ( $I_{\delta-ms}$ ), j) the Morisita index for reproductive success ( $I_{\delta-rs}$ ) and k) the Bateman gradient ( $\beta_{ss}$ ).

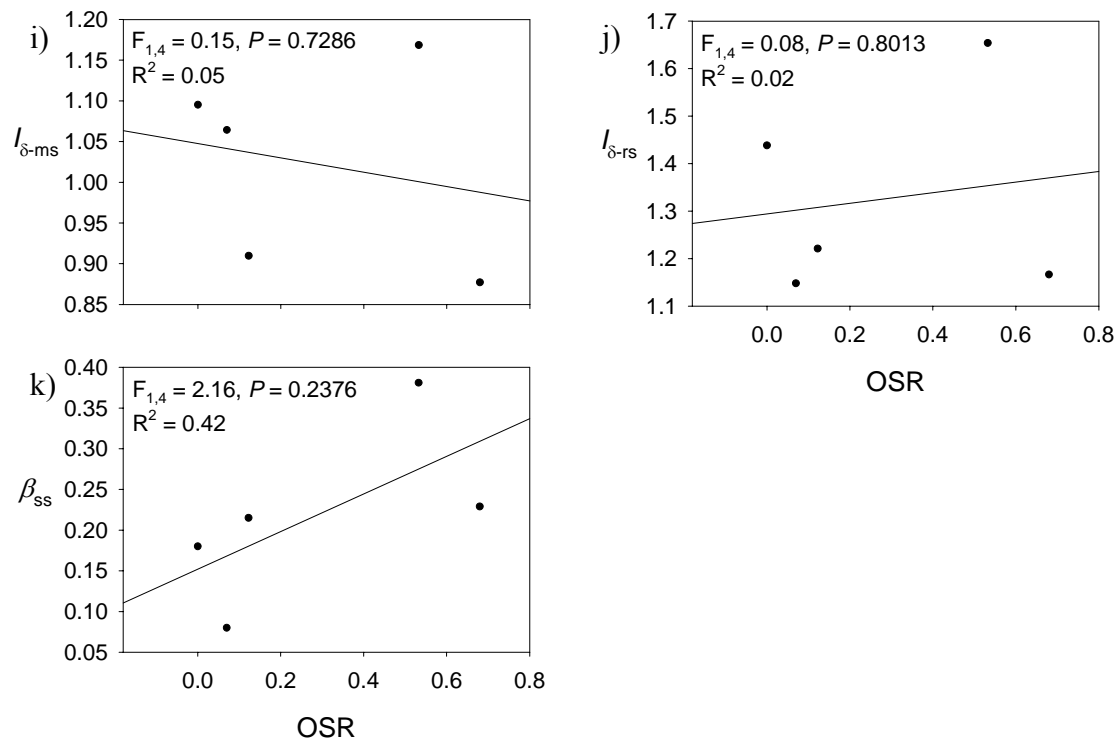


Figure 5.5 continued.

### *Dependence of genetic mating system correlates on the OSR*

The natural variation in the genetic mating system between sites and sampling times allowed for the exploration of the relationship between OSR and estimates of the male genetic mating system. All estimates of the male genetic mating system were not significantly correlated with OSR except the variance in reproductive success ( $\sigma^2_{rs}$ ) which was significantly correlated with OSR ( $F_{1,4} = 10.65$ , 4 d.f.,  $P < 0.05$ ). Estimates of the mean mating and reproductive success decreased with an increasing estimate of the OSR during time of collection. Variance, variance-based estimates ( $I$ ,  $I_s$ ,  $\beta_{ss}$ ) and the index for resource monopolization ( $Q_{ms}$ ,  $Q_{rs}$ ) increased with increasing OSR whereas the Morisita index ( $I_{\delta-ms}$ ,  $I_{\delta-rs}$ ) appeared to have no relationship with the OSR.

### **Discussion**

This study is the first to document temporal variation in the genetic mating system among natural populations of the pipefish *S. typhle*. A significant increase in the male mating and reproductive success and the estimates of the three Bateman's principles between the first and second pregnancy in the Tjuvsund site demonstrate that male *S. typhle* experience different sexual selection regimes during the course of the summer breeding season. This study also is the first to show a positive relationship between the operational sex ratio and Bateman's principles in natural populations thereby demonstrating the flexibility and usefulness of their application to natural systems.

A major goal of this study was to document the effects of a changing OSR on the genetic mating system of *S. typhle*. The expectation was that an increase in the OSR towards female-bias during the second pregnancy would produce stronger female-female competition and affect the number of mates and embryos of males similar to the measured response in mesocosm experiments that artificially manipulated sex ratios (Jones et al. 1999, Jones et al. 2004, Jones et al. 2005, Mills et al. 2007). In these experiments, individuals placed in equal sex ratios experienced lower sexual selection and these treatment subsequently had lower estimates of the opportunity for selection, the opportunity for sexual selection and the Bateman gradient. Skewed sex ratio treatments, on the other hand, resulted in systematic increases in variance-based measurements of the genetic mating system of the non-limiting sex while having a minimal effect on these measures in the limiting sex (Jones et al. 1999, Jones et al. 2004, Jones et al. 2005, Mills et al. 2007). In the case for *S. typhle*, males limit reproduction of females by only being available to mate during the initiation of the mating season and after giving birth. Therefore one would expect that highly skewed OSRs would affect females the greatest and males to a lesser extent. In this study there is an increase in the standardized variance in mating and reproductive success between the first and second pregnancy in the Tjuvsund site. This surprising result suggests that the males experience higher sexual selection in the second time period contrary to expectations and that the male genetic mating system is more flexible in *S. typhle* than previously hypothesized.

Despite differences in the genetic mating system estimates, neither the number of mates nor the number of embryos per mated male differed between the two samples

from Tjuvsund. One explanation why we might not see a difference between the first and second pregnancy is that the Tjuvsund site had a male-biased adult sex ratio. Males may therefore experience an OSR closer to 0.5 during the second pregnancy than other populations might experience. Similarly, female behavior in terms of the female reproductive contribution does not differ in samples with variable OSRs and the mean values of female reproductive contribution appear to be primarily a function of female body length. We know from behavioral studies that larger females contribute more eggs per copulation than do smaller females (Berglund et al. 1986) and that both morphological and behavioral traits may vary between sites separated by as little as 4 km for this species (Robinson-Wolrath 2006). Given the significant difference in adult female body size between sites, this may help to explain variation in female reproductive contribution between sites and times. It is difficult to disentangle the effect of the OSR on mate competition based on this study alone and further investigation of this relationship is clearly warranted.

A second goal of this study was to explore the relationship between the OSR and different methods for estimating the genetic mating system. Despite similarities in the number of mates and number of embryos per mated male at each sampling time, estimates of the genetic mating system showed significant differences between sites and times. All measures of the genetic mating system consistently showed the greatest estimates for mating and reproductive success during the 2<sup>nd</sup> pregnancy of the Tjuvsund site in 2005, suggesting that sexual selection acts most strongly on males during the second mating period. However caution should be exercised with this result since

perceived differences in the genetic mating system are a direct result of the inclusion of non-mated males in the estimate. In *S. typhle*, the pregnancy in males can last between 4-6 weeks (Berglund et al. 1989). Because of the length of the pregnancy and fluctuations in the OSR, individuals may experience a different OSR at the time of sampling than that experienced at the start of the mating period. As a result of this lag in OSR, a temporal disconnect between the OSR at the time of collection and the time prior to the first pregnancy may exist in this species. Therefore estimates of the genetic mating system that rely on the estimate of unmated individuals may be vulnerable to errors based on temporal sampling of mates as unmated individuals may have a disproportionate affect on the variance in mating and reproductive success in this species.

Variance-based measures of the male genetic mating system were positively correlated to the OSR although only the variance in reproductive success was significantly correlated with OSR. While the OSR may predict the outcome of competition an individual experiences at a particular point in time, it may not be directly correlated with the ecological and demographic conditions that males and females are subjected to at the time of copulation. For example, species with high costs to reproduction such as long gestation times, long periods of parental care or low reproductive turnover act to increase the length of time an individual can be considered able to mate (Clutton-Brock & Vincent 1991, Kvarnemo & Ahnesjö 1996). This issue of temporal variation in the OSR is likely not a problem for species that do have negligible costs to reproduction as the number of unmated individuals would be heavily

influenced by the OSR and ASR (Kvarnemo & Merilaita 2006). A recent simulation study has provided theoretic evidence that the OSR is influenced by the distribution of matings in time when the male mating was limited by parental care. A delay in the effect of ‘times-out’, or the time a male is unavailable for mating, increases with increasing mate variation (Kvarnemo & Merilaita 2006). Simulation-based modeling of this nature may help to fill in the gaps of knowledge between the response of mating system estimates to a fluctuating OSR.

This study highlights the inherent problems with measuring sexual selection in natural populations. For smaller populations where a high proportion of mates can be captured such as the Trinnhålet site in this study, one may be able to reconstruct and compare the genetic mating system of both sexes with reasonable certainty. However, in large populations, such as the Tjuvsund site, where the collection of a large proportion of individuals and their actual mates is unlikely, the reconstruction of the genetic mating system is not possible for the sex of interest. Often times collections made in the field are the only time the site is visited and therefore estimates such as the OSR and ASR may be subject to temporal stochasticity that does not accurately reflect the true conditions under which mating took place. Despite these obstacles, the measurement of sexual selection can and should remain a primary goal of sexual selection research and poses an interesting methodological and theoretical challenge for future studies.



## CHAPTER VI

### CONCLUSIONS

Behavioral ecology has capitalized on the application of powerful molecular markers to investigate patterns of parentage in natural populations (Avis 2004). These tools have added a statistically rigorous component to a growing theoretical framework for testing sexual selection theory and have opened new doors to the study of genetic mating systems (Arnold 1994, Arnold & Duvall 1994, Shuster & Wade 2003). Although there has been much effort put forth to understand many aspects of mating system evolution, large scale tests of mating system theory over broad spatial and temporal scales are generally lacking. Two major stumbling blocks contribute to our lack of understanding of this phenomenon. First, mating systems are assumed to be fixed for many species as most studies that concern mating systems take place in one population at one geographic locale at one particular time. Second, we have a good understanding of factors that are responsible for mating system behavior such as the direction and intensity of sexual selection on each sex, yet we lack a basic understanding of environmental factors extrinsic to the mating system that may influence mating behavior. Therefore, the main focus of this dissertation was to contribute significantly to the understanding of how genetic mating systems are organized with respect to temporal and geographic variation.

In order to test more specific hypotheses about geographic variation in the genetic mating system of the pipefish species *Syngnathus floridae*, it was necessary to first investigate whether or not populations of *S. floridae* represented different

populations of the same species rather than multiple collections of subspecies. By doing so, it diminishes the possibility that genetic mating systems are regulated by the genetic architecture of populations. In **CHAPTER II**, microsatellite data revealed that populations sampled from the Atlantic and Gulf of Mexico that populations of *S. floridae* follow a strong isolation by distance trend and show some regional structuring according to ocean basins. Mitochondrial DNA analysis, on the other hand, provided support that Atlantic and Gulf of Mexico populations share gene flow between ocean basins, although mechanisms for such gene flow remain elusive and would likely require more samples throughout the entire geographic range of *S. floridae*. Because these results provide little support for deep phylogenetic splits between ocean basins, it is fair to conclude that differences in the genetic mating system between populations are not likely due to any phylogeographic relationship. Rather, differences between populations are likely the result of particular environmental and demographic factors that are specific to each site and each time period sampled.

**CHAPTER III** investigates the genetic mating systems of two geographically distinct populations of *S. floridae* from the Atlantic Coast of Virginia and the Gulf Coast of Florida. This species is characterized by polygynandry and male pregnancy. The results of parentage analysis of pregnant males revealed significant inter-population variation in mating and reproductive success. Estimates of the opportunity for selection, the opportunity for sexual selection and the Bateman gradient were higher among males in the Florida population than in the Virginia population, suggesting that sexual selection on males is stronger in the Florida population. The Virginia population is larger and

more dense than the Florida population suggesting that population demographics may be one of many causal factors shaping inter-population mating patterns. This study also provides evidence that the adult sex ratio, operational sex ratio, population density and genetic mating system of *S. floridae* may be temporally stable over time scales of a month in the Florida population. Overall, these results show that this species is a good model for the study of mating system variation in nature and that Bateman's principles may be a useful technique for the quantitative comparison of mating systems between populations. Moreover, the results of this comparison set the stage for further comparison of environmental and demographic factors that may influence the genetic mating system among several more populations of *S. floridae* (**CHAPTER IV**).

In **CHAPTER IV**, the investigation of geographic variation in *S. floridae* is expanded to include three additional populations including North Carolina along the Atlantic coast and Tampa Bay, Florida and Port Aransas, Texas along the Gulf of Mexico coast. The addition of these populations allowed for the partitioning of the relative associations of specific demographic and environmental factors on the mating system measures of the female reproductive contribution and mean mating success, mean reproductive success, the opportunity for selection, the opportunity for sexual selection and the Bateman gradient of males. Populations of *S. floridae* differed in body length of males and females and female body depth. Populations also varied in demographic criteria such as adult and operational sex ratios, population density and population size and experienced dissimilar environmental regimes such as temperature, salinity, turbidity and seagrass habitat. Mean yearly water temperature and temperature

measured in the field explained a high proportion of the variance in female reproductive contribution, male mating success, the opportunity for selection and the Bateman gradient. This result is likely due to the strong correlation of temperature over the potential reproductive rates of males and females. Total seagrass biomass was also shown to be significantly correlated with the female reproductive contribution and played a small but significant role in the variance experienced in the male opportunity for selection. Demographic processes such as the adult sex ratio and the adult population size and density also significantly contributed to variation in the mating system of *S. floridae*. The results of this study provide the first conclusive evidence that mating system correlates and Bateman's principles are correlated with specific ecological criteria. The significance of this result is that both demographic and environmental factors play a major role in shaping the genetic mating system, whereas morphological attributes such as body size, which are important in regulating specific mate choice behaviors in pipefishes, appear not to have a major influence on the mating system over a broad geographic scale.

The purpose of this study in **CHAPTER V** was to investigate the extent to which a changing operational sex ratio influences the genetic mating system in natural populations of the broad-nosed pipefish, *Syngnathus typhle*. The questions posed during the study were: (1) does an increasingly female-biased operational sex ratio affect the genetic mating system? And (2) do measures of the genetic mating system such as the mean mating success, mean reproductive success, the three Bateman's principles, the opportunity for selection, the opportunity for sexual selection, and the Bateman gradient,

and two additional measures of the genetic mating system the Morisita Index and the index for resource monopolization correctly predict and respond to changes in hypothesized mate competition as measured by the operational sex ratio? The results of this study show a significant increase in the male mating and reproductive success and the estimates of the three Bateman's principles between the first and second pregnancy concomitant with an increase in the female-bias in the operational sex ratio. This study is also the first to show a positive relationship between the operational sex ratio and variance-based measures of the male genetic mating system such as Bateman's principles in natural populations thereby demonstrating the flexibility and usefulness of their application in natural systems. However, while the operational sex ratio may predict the outcome of competition an individual experiences at a particular point in time, it may not be directly correlated with the ecological and demographic conditions that males and females are subjected to at the time of copulation.

Taken on the whole, these studies detailed herein significantly contribute to the growing empirical support of a quantitative approach to mating system organization. These studies are revolutionary in the sense that these are the first large scale tests of mating system theory conducted in natural populations and over broad geographic and temporal scales. Because these studies rely on a whole systems approach, these studies take the next logical step in filling the gap of knowledge as to how ecological factors may affect mating systems. These studies also point out specific flaws and areas of improvement for the broad-scale application of these techniques to natural systems. Furthermore, these studies also provide data to help build predictive models how mating

systems may respond to changing environmental conditions and global climate change. In addition, given the unusual nature of pipefish mating systems, these studies also provide novel insight on the evolution of pipefish mating behavior and mating systems in general. Thus results from these studies significantly enhance our understanding of how mating systems are organized over broad environmental gradients and temporal/spatial scales.

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